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EDITORIAL

Neurological Rehabilitation of Stroke Patients by Means of a Robotically Assisted Brain Controlled Interface

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Stroke is considered to be the single leading cause of adult disability worldwide. According to a census provided by the World Health Organization, every year, as many as 15 million people suffer from stroke. Of these, 5 million die, and another 5 million become permanently disabled. In Malaysia, there are approximately 10,000 new stroke cases each year; 70% of patients who recover stop taking part in social activities, 30% need assistance coping with daily life, and 15% die within a month (1). Strokerelated motor deficits can be rehabilitated to a greater degree than deficits resulting from other neurological disorders and traumatic brain injuries. Presently, neurological physiotherapy is the most common treatment to enhance mobility as well as sensory faculties impaired by stroke.

The advent of digital technology, together with the rapid growth of signal processing techniques, has resulted in the creation of mechatronic devices that are capable of performing new rehabilitation schemes. One of the emerging technologies is the brain-computer interface (BCI) or brain-machine interface (BMI). This new computer-aided apparatus attempts to decode the brain's electrical activities and translate them into control signals for activating external devices. Recent experiments on stroke survivors and people paralysed by spinal cord injuries and amyotrophic lateral sclerosis provide evidence that BCI promotes cortical plasticity in which the brain reorganises around damaged cortical areas, thereby recovering the functions of those areas (2). It has also been reported that the combination of robotic rehabilitation and BCI systems has the potential to outperform classical therapies in measuring and restoring lost motor skills (3). In any BCI system, the first step is to capture signals containing information about the patient's intent. Among many available recording modalities, electroencephalogram (EEG) is arguably the most common and practical technique for capturing brain activity. This is because, in the electroencephalographic method, electrodes are safely and noninvasively placed on the scalp as opposed to being placed into the dura or cortical layers of the brain. The only drawback is that EEG signals are bandlimited, resulting in a limited temporal resolution and frequency range. Generally, EEG signals from the surface of the brain do not contain significant frequency content above 70 Hz. However, this does not pose a significant problem for BCI because EEG responses to imaginary motions and mental tasks are dominated by event-related synchronisation (ERS) and desynchronisation (ERD) of alpha (812 Hz) and beta waves (1825 Hz) (4). Mu-rhythms, which are closely related to cortical motor planning, falls within the alpha band. In most healthy adults, the mu-wave is attenuated whenever voluntary, passive, or reflexive movements are performed or imagined. This desynchronisation corresponds to activation of the sensorimotor areas and reflects increased cellular excitability in the brain during cortical information processing. In contrast, the mu-rhythm (particularly the alpha component) is synchronised somatotopically when the brain is idle or at rest. As a result, the EEG amplitude increases during motionless rest. The attenuation (ERD) and amplification (ERS) of alpha waves form the basis of BCI control and machine interfaces. Thus, a severely paralysed patient can use mu-rhythms to move a cursor on a computer screen or establish simple communication like "yes" or "no" answers. This strategy is relatively straightforward and has already been the focus of a considerable body of research. The more complex task is to use these signals that allow a disabled person to communicate and translate them into actions that reflect his or her intent. This strategy is based on the hypothesis that repeated activation and deactivation of brain signals restores motor function and induces brain plasticity (2).

To test this hypothesis, it is necessary to have a controlled environment in which the patient attempts to perform a specific imagery motion while a robotic arm provides control and aids completion of the motion. This has been the model for the design and construction of the BCI-assisted robotic arm system for rehabilitation of stroke survivors. Figure 1(a) shows a class 3 stroke patient

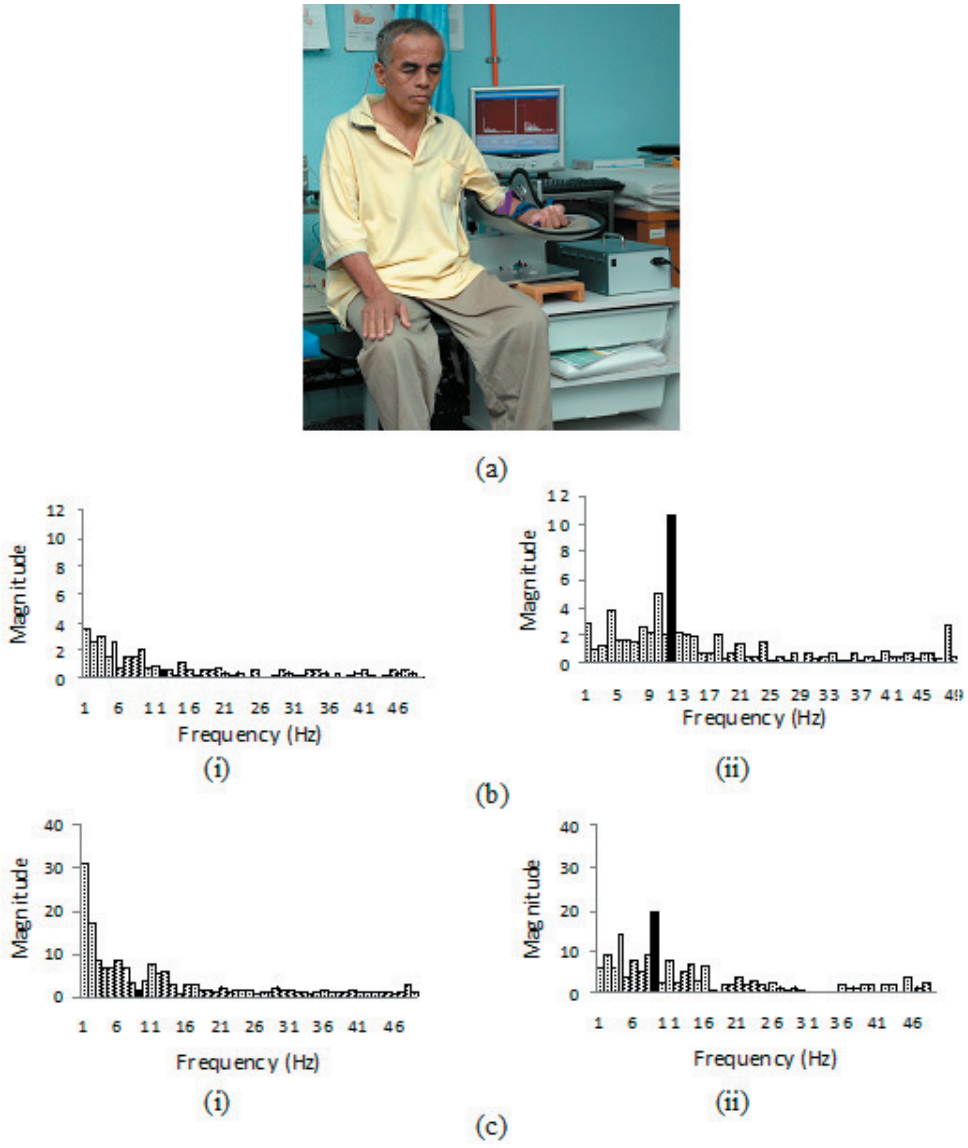

Figure 1: Prototype BCI system for adaptive therapies utilising 1-dimension shoulder-elbow movements. (a) An overall view of the system. (b), (c) EEG patterns recorded from healthy and stroke subjects, respectively. These signals were recorded during voluntary movements or imagery motion (i) and motionless rest (ii).

with EEG probes attached to the cortical areas of the scalp, using the robotic arm. The basic goal of the design is to capture alpha waves as accurately as possible while minimising noise, especially artefacts due to electromyographic muscle movement and other environmental interference. With this goal in mind, we designed the 10th order Sallen-Key band-pass filter, which filters out unwanted components and allows only selected frequencies to pass through. The overall gain of the filter is set to 1239, and the bandwidth is fixed to 30 Hz. This design spatially attenuates unwanted noise by 60 dB while enhancing the signal-to-noise ratio by more than 100 dB. The filtered, amplified signal is sampled at 100 kHz and digitised using a 12-bit analogue-to-digital (A/D) converter. The signal is then fast Fourier transformed to produce a power spectral distribution of the signal. Altogether, four EEG probes are used in the experiments comprising a healthy subject and a stroke patient. Figure 1(b) and (c) show spectra recorded from channels corresponding to a healthy subject and a stroke subject, respectively. In these figures, (i) indicates the spectrum when the subject is awake, and (ii) indicates the spectrum when the subject is at rest. Clearly, the amplitude of the mu-rhythm generally decreased when both subjects voluntarily moved their arms or performed emotion movements. However, the EEG pattern, particularly the 10 Hz alpha wave, increased remarkably when they

were at rest. Expectedly, the percentage increase in amplitude was much higher in the healthy subject compared to the stroke patient. These patterns,which are associated with the voluntary and imagery modulations of the mu-rhythm, provide unique fingerprints corresponding to subjects' motor intents. These patterns allowed us to devise
a strategy for translating imagination of movements into robotic commands.

The system offers a communication interface that is matched with a stroke patient's residual motor disabilities. We believe that such a prototype could facilitate translation from pre-clinical demonstrations to clinically useful applications, indirectly proving the hypothesis that adaptive therapies promote brain plasticity.

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REVIEW ARTICLE

Cytogenetics: Past, Present And Future

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Abstract

Fifty years have elapsed since the discovery of the number of human chromosomes in 1956. Newer techniques have been developed since then, ranging from the initial conventional banding techniques to the currently used molecular array comparative genomic hybridisation. With a combination of these conventional and molecular techniques, cytogenetics has become an indispensable tool for the diagnosis of various genetic disorders, paving the way for possible treatment and management. This paper traces the history and evolution of cytogenetics leading up to the current state of technology.

Keywords: *Cytogenetics, Chromosomes, medical sciences*

History of cytogenetics

It was the Swiss botanist Nageli who first described thread-like structures in the nuclei of plant cells in the 1840s, and what he called "transitory cytoblasts" are now known as chromosomes. Later, in 1888, Waldeyer coined the term "chromosome" after staining techniques had been developed to make them more discernible (chromos = Greek for colour; soma = Greek for body). Cytogenetics is the study of the structure and properties of chromosomes, their behaviour during somatic cell division during growth and development (mitosis), and germ cell division during reproduction (meiosis), as well as their influence on phenotype. Cytogenetics also includes the study of factors that cause chromosomal changes (1).

Initially, it was difficult to determine the diploid number of mammalian species because the chromosomes were crowded in metaphase. In the 1950s, several technical improvements, such as the addition of colchicines to arrest cells in metaphase and the use of hypotonic solution to obtain better chromosome spreads, were made (2,3,4). In 1956, the diploid number of chromosomes in man was established as 46 (5), and the peripheral leucocyte culture method of Moorehead et al. (6) was adopted by many cytogeneticists. It was then possible to describe correctly the normal human chromosome number and chromosome abnormalities. This enabled detection of numerical chromosome aberrations like trisomy 21 in Down syndrome (7), 45, X in Turner syndrome (8), 47, XXY in Klinefelter syndrome (9), trisomy 13 (10), trisomy 18 (11), and Philadelphia chromosome in a patient with chronic myeloid leukaemia (12). It was also reported that cells cultured from amniotic fluid could be used to determine the chromosome content of the foetus (13). The metaphase chromosomes were classified into seven groups based on the Denver classification (1960) (14), with revisions at the London Conference (1963) (15) and the Chicago Conference (1966) (16). Jau-hong Kao et al. (17) described a chromosome classification based on the band profile similarity along the approximate medial axis.

Advent of banding techniques

Caspersson et al. (18) discovered one of the first chromosome banding techniques (Q-banding), which involved staining chromosomes with a fluorochrome, such as quinacrine mustard or quinacrine dihydrochloride, and examining them with fluorescence microscopy. This technique, however, was less than optimal for routine studies, as the fluorescent staining quickly quenched. Hence, several other banding techniques were developed, for example, G-, R-, C- and NOR banding, each having its own specific properties and applications (19).

In due course, G-banding, produced by staining the chromosomes with Giemsa solution, became the most frequently used method (20). This gave better resolution than Q-banding, allowed permanent preparations, and did not necessitate the use of fluorescence microscopy. Pardue and Gall (21) first reported C-bands in 1970, when they discovered that the centromeric region of mouse chromosomes is rich in repetitive DNA sequences and stains darkly with Giemsa. C-bands localise in the heterochromatic regions of chromosomes. Many chromosomes have regions that differ among individuals but have no pathological importance. These polymorphic regions can be visualised optimally with C-band methods. C-banding is also useful to show chromosomes with multiple centromeres, to study the origin of diploid molar pregnancies and true hermaphroditism, and to distinguish between donor and recipient cells in bone marrow transplantation. Nucleolar organising region (NOR)-banding is a technique that stains NORs of chromosomes (22). These regions are located in the satellite stalks of acrocentric chromosomes and house genes for ribosomal RNA. Goodpasture et al. (23,24) developed a simple silver nitrate staining technique for NOR-banding that is useful in clinical practice to study certain chromosome polymorphisms, such as double satellites.

However, the resolution of chromosome studies remained relatively limited because the total number of bands produced on metaphase chromosomes was low, and it was difficult to detect rearrangements involving small portions of chromosomes due to excessive condensation. This situation was improved by the development of high-resolution banding by Yunis (25), which was achieved by synchronising the lymphocyte cultures and obtaining more cells in pro-metaphase or even prophase. High resolution cytogenetics provided greater precision in the delineation of chromosomal breakpoints and assignment of gene loci than earlier techniques could since analysis of late prophase sub-banding reveals more than twice the number of bands seen at metaphase (26). By applying this technique, several well-known clinical syndromes, like Prader-Willi and Angelman syndromes with deletions at the proximal long arm of chromosome 15, Smith-Magenis and Miller-Dieker syndromes with (different) deletions in the short arm of chromosome 17, and DiGeorge/Velo Cardio Facial (VCF) syndromes with deletions in the long arm of chromosome 22, could be linked to small chromosome aberrations, and the concept of the micro-deletion or contiguous gene syndrome was born (27).

The choice of banding technique

For routine analysis, however, the G-banding technique using trypsin and Giemsa became the most accepted worldwide (28). The banding pattern enabled the detection of various structural aberrations like translocations, inversions, deletions, and duplications in addition to the well-known numerical aberrations. This led to the cytogenetic investigation of healthy family members of known carriers and of couples suffering from repetitive spontaneous abortions (29).

Specialised techniques to visualise chromosomes

Sister Chromatid Exchange (SCE)

SCE enabled visualisation of interchanges between brightly and dully fluorescent segments of sister chromatids. This was made possible by incorporating BrdU (in place of thymidine) into replicating cells for 2 cell cycles. The biologic importance of SCEs is uncertain, but some mutagens and carcinogens increase their frequency (30). It has been noted there is an increase of SCE in patients with ankylosing spondylitis (31), in smokers (32), in women after exposure to biomass fuels (33), and in patients with carcinoma of the cervix uteri (34).

Fragile sites and chromosome breakage

Gaps that are consistently seen at the same chromosome locus are called fragile sites. Some fragile sites are associated with specific medical conditions such as Fragile X Syndrome, which is associated with a fragile site at Xq27.3 (35,36). All humans experience increased chromosome breakage when exposed to cytotoxic agents, but in certain autosomal recessive disorders, the inability to repair DNA is associated with certain kinds of chromosome damage (37). The detection of a fragile site in a patient often causes concerns due to its potential significance, and it must be followed-up properly with genetic counselling (38).

Molecular cytogenetics

Fluorescent in situ Hybridisation (FISH)

In 1986, Pinkel et al. (39,40) developed a method to visualise chromosomes using fluorescent-labelled probes called fluorescent in situ hybridisation (FISH). FISH technology permits the detection of specific nucleic acid sequences in morphologically preserved chromosomes, cells, and tissues. These techniques are useful in the work-up of patients with various congenital and malignant

neoplastic disorders, especially in conjunction with conventional chromosome studies. Using FISH, cytogeneticists can detect chromosomal abnormalities that involve small segments of DNA if their probe is situated, fortuitously or by design, in the affected chromosomal segment (41). FISH can be used to establish the order of DNA clones relative to bands, naturally occurring breakpoints, and other clones. Even more importantly, FISH permits karyotype analysis of nuclei in non-dividing cells. FISH has been used for the detection of t(2;5) (p23;q35) translocation in anaplastic large-cell lymphoma (42), for minimal residual disease in haematopoietic stem cell assays from peripheral blood stem cells of acute myeloid leukaemia (AML) patients with trisomy 8 (43), and for analysing chromosomal abnormalities of tumours in children (44).

Spectral Karyotyping (SKY) and Multicolour FISH (M-FISH)

FISH paved the way for a more powerful technology called spectral karyotyping (SKY) or multicolour FISH (M-FISH). M-FISH allows all of the 24 human chromosomes to be painted in different colours. By making use of various combinations and concentrations of fluorescent dyes, it is even possible to give every single chromosome a different colour (SKY), which can be of particular use when dealing with complex aberrations often associated with various types of solid tumours. SKY or M-FISH enables production of chromosome-specific 'paints'. Fluorochromes are combined to produce 24 colour combinations, one for each chromosome (45), resulting in multicolour analyses. SKY paints the entire chromosome in the same colour, whereas M-FISH uses various fluorescence dyes to represent different painting probes at the same time. This offers the simultaneous presentation of all 24 different human chromosomes with a single hybridisation. SKY and M-FISH have proven to be extremely useful in detecting translocations and other complex chromosomal aberrations. The main applications for M-FISH have been in solid tumours, which are often characterised by complex karyotypes, and in AML and acute lymphoblastic leukaemia (46).

Comparative Genomic Hybridisation (CGH)

FISH investigations have proven to be advantageous in many ways, but they are time consuming because preparations must be hybridised and then microscopically analysed. These problems led to the development of a variation of FISH called CGH (47). Later, a further improvement was developed an array based on comparative genomic hybridisation (48, 49). CGH does not require the preparation of metaphase chromosomes from cells. Instead of hybridising a labelled probe to human chromosomes on a slide, it is now possible to print thousands of different and well-characterised probes on a glass slide. The array-CGH is even more promising than the conventional CGH (50). Array-CGH is the equivalent of conducting thousands of FISH experiments at once, and it provides better quantification of copy number and more precise information on the breakpoints of segments that are lost or gained than does conventional CGH. It is faster and has a better resolution than available molecular cytogenetic tools (51).

Cancer cytogenetics

The involvement of chromosomal aberrations and the deviation from the normal copy number of a given chromosome (aneuploidy) in tumours have long been known. For example, balanced chromosomal translocations can have oncogenic effects through the production of fusion proteins. In the case of chronic myelogenous leukaemia (CML), 95% of the cases harbour a translocation between chromosomes 9 and 22, which results in the formation of what is commonly referred to as the Philadelphia chromosome. Glivec is the main treatment for CML, which is a biological treatment that targets a protein made by CML cells. Glivec is a type of growth blocker, called a tyrosine kinase inhibitor (TKI). Tyrosine kinases are a group of proteins that cells use to signal to each other to grow. This drug stops the messages from these proteins getting to cancer cells and thus interferes with their growth. Routine conventional cytogenetics is used to monitor the progress of this treatment. In most people, there is a major drop in the number of cells that carry the Philadelphia chromosome. As a consequence, the implementation of cytogenetic analyses, at least at diagnosis, is mandatory for analysing the outcomes of many clinical trials, and it can also be used to stratify patients for different types of therapy (52,53).

The current situation and the future

Currently, cytogeneticists are developing molecular approaches for deciphering the structure, function and evolution of chromosomes. Conventional cytogenetics using regular banded chromosomal analysis remains a simple and popular technique to get an overview of the human genome. Routine banded karyotype

analysis can now be combined with M-FISH and various other molecular techniques, leading to more precise detection of various syndromes in children. The combination of CGH (54) with multicolour FISH was seen from the beginning to be a powerful combination for 7characterising complex karyotypes (55,56,57,58). More recently, microarray-based formats using large insert genomic clones, cDNAs or oligonucleotides have replaced metaphase chromosomes as DNA targets (49), providing higher resolution and the ability to directly map the copy number changes to the genome sequence. In other words, chromosomal abnormalities exist as nature's guide to the molecular basis of many unexplained human disorders. Thus, techniques of cytogenetics are bound to continue to be indispensable tools for diagnosing genetic disorders and indicating possible treatment and management.

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SPECIAL COMMUNICATION

The Role Of Cells, Neurotrophins, Extracellular Matrix And Cell Surface Molecules In Peripheral Nerve Regeneration

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Abstract

Wallerian degeneration is a complicated process whereby axons and myelin sheaths undergo degeneration, and eventually are phagocytosed by macrophages and Schwann cells following nerve damage. Schwann cells proliferate and the endoneural tubes persist. In addition, neurotrophins, neural cell adhesion molecules, cytokines and other soluble factors are upregulated to facilitate regeneration. The important role of cellular components, neurotrophins, and extracellular matrix components, including cell surface molecules involved in this regenerative process, is highlighted and discussed in this review.

Keywords: *cell surface molecules extracellular matrix, nerve growth factors, nerve regeneration, Schwann cells, neurosciences*

Introduction

Various cellular components such as Schwann cells, macrophages, fibroblasts, and mast cells are recruited during peripheral nerve regeneration. In addition, research has shown that neurotrophins, extracellular matrix components and cell surface molecules are also vital for successful nerve regeneration. Available research data on these components are discussed below.

Schwann cells

Schwann cells, macrophages and mast cells are the three main cell types that are involved in peripheral nerve regeneration. The importance of Schwann cells in peripheral nerve regeneration was demonstrated in a study carried out by Hall (1). The investigator compared the regeneration that occurred in the cellular and acellular autografts using electron microscopy. The study showed that neurites grew into fresh autografts and rapidly re-establish functional relationships with the Schwann cells lying in bands of Büngner within the graft, however penetration of acellular grafts was less efficient. In another study carried out by the same author (2), following mitomycin C treatment, an anti-mitotic agent known to arrest Schwann cell division after nerve injury, very few neurites grew into a cellular nerve autografts, suggesting that neurite outgrowth from the proximal stump is dependent upon active Schwann cell participation.

There are a variety of mitogenic factors that can stimulate Schwann cell proliferation. These factors include the extracellular matrix components, myelin debris, macrophage-derived cytokines and Schwann cell-Schwann cell signalling (3). In addition, axon-derived signals have also been shown to cause Schwann cell proliferation. One component of the axon-derived signal that has been well characterised is the neuregulin family (4). One of the family members, neuregulin-1 (NGR1) is a survival factor for immature Schwann cells in the developing peripheral nerve. NGR1 mRNA is reported to be present at high levels in the cell bodies of the adult sensory and motoneurons (5).

Based on in vitro studies carried out by Mahanthappa et al. (6) on the effects of recombinant human glial growth factor 2 (rhGGF2), the investigators proposed that the secretion of neuregulin from the growth cones of the regenerating axons may set up a concentration gradient within a local environment. According to their hypothesis, the neuregulin serves as a chemoattractant, so that Schwann cells migrate towards the site of injury where the neuregulin

concentration is high and this stimulates the Schwann cells to proliferate and also secrete trophic factors to support axon regeneration.

Apart from axon-derived signals to regulate Schwann cell survival, investigations carried out by Jessen and Mirsky (7) suggest the presence of autocrine survival loops which provide an axon-independent way of surviving. Their in vitro study has shown that Schwann cells grown at a sufficient density to achieve a concentration of secreted factors are capable of surviving in the absence of neuronal signals. In addition, it has been reported that the Schwann cells in the distal stump also upregulate the expression of both neuregulins and the erbB2 neuregulin receptor (8). These observations indicate that Schwann cells might be able to survive even when the axons are damaged.

Additionally, mitogenic signals can also be released by damaged Schwann cells. Nerve injury that leads to Schwann cell damage releases intracellular ciliary neurotrophic factors (CNTF), which then signals via axonal leukaemia inhibitory factor (LIF) receptors to induce Reg-2 which is a Schwann cell mitogen (9). Additional mitogenic signals for Schwann cells can also come from macrophages which secrete platelet-derived growth factor (PDGF), transforming growth factor-ß (TGF-ß) and fibroblast growth factors (FGF-ß) which are all mitogenic for Schwann cells (10). On the other hand, axotomised Schwann cells can downregulate expression of myelin protein genes (such as myelin-associated glycoprotein, myelin basic protein and PO) and upregulate gene expression of low affinity neurotrophin receptor p75, GAP43, GFAP, neurotrophins like NGF and BDNF, the neu receptor c-erbB2, cell-cell adhesion molecules Neural-Cell Adhesion Molecule (N-CAM) and L1, extracellular matrix molecules like laminin, J1/tenascin and fibronectin. Following establishment of functional contact with axons, Schwann cells also upregulate expression of ß4 integrin, laminin B1 and B2 chains (11).

Clearly, from the studies reviewed above, the Schwann cells appear to be essential for peripheral nerve regeneration. However, macrophages and mast cells are also important cellular components, and will be discussed next.

Macrophages

Major macrophage invasion usually occurs around the third day following nerve injury. The mechanism by which this invasion occurs is not clearly understood, but it is thought that chemokines such as macrophage chemotactic protein-1 (MCP-1) and macrophage inhibitory protein-1a (MIP-1a), are produced by Schwann cells or resident macrophages in response to nerve injury (3). Macrophages deliver a variety of important molecules including basic fibroblast growth factor, granulocycte-macrophage colony stimulating factor, transforming growth factor alpha (TGF-a), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), and interleukin-8 (12).

Mast cells

Mast cells are important cells involved in the inflammatory response. Mast cell degranulation and the release of histamine may be important in opening the blood nerve barrier after nerve injury. Mast cells also contain growth factors, proteolytic enzymes, tumour necrosis factor alpha (TNF-a), interleukins and cytokines which may be released following nerve injury (12).

Neurotrophins

The five known neurotrophins are named nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrphin-4/5 (NT-4/5) and neurotrophin-6 (NT-6), and some of them may have important functions in peripheral nerve regeneration. These neurotrophins act via receptor tyrosine kinases (RTK): NGF interacts with trkA; BDNF and NT-4/5 interact with trkB; NT-3 interacts with trkA, trkB and trkC; and all of these neurotrophins (except NT-6 which is not fully characterized yet) interacts with receptor p75 (13). Of these, NGF, NT-3 and BDNF show beneficial effects on the survival and regeneration of primary sensory neurons in the dorsal root ganglion (DRG) and motoneurons in the spinal cord (5).

The roles of neurotrophins were confirmed in knockouts. Studies by Snider and Silos-Santiago (14), showed that both the trkA-/- and trkC-/- receptor mutant mice have striking neurological deficits which can be ascribed to neurons in the DRG. The trkA-/- animals showed massive DRG neuron loss in the lumbar ganglia, and almost all unmyelinated and 50% of myelinated axons were lost. The trkC-/- animals showed writhing movements of the extremities that were noticeable within the first few days of life, reduced size of the DRG, a modest reduction in the DRG neuron number, depletion of large calibre axons in the dorsal roots, and reduction in area of the dorsal column. On the other hand, trkB-/- animals had less profound effects in the DRGs observed. Most of these neurotrophins are target-derived, however,

the localization of BDNF in a population of DRG neurons suggests the additional possibility that BDNF could act in a paracrine or autocrine manner to mediate neuronal survival (15).

In relation to nerve injury, the importance of these neurotrophins can be seen in studies carried out by Verge et al. (16). To assess the role of neurotrophins in vivo, the authors investigated the effects of intrathecal administration of NGF and NT-3 on DRG following unilateral sciatic transection in the adult rats. Their result showed both NGF and NT-3 were able to counteract axotomy induced changes in the DRG neurons. In another study, Sterne et al. (17) reported that NT-3 delivered locally via fibronectin mats enhances peripheral nerve regeneration. Similar enhancement of peripheral nerve regeneration was also reported following delivery of CNTF following sciatic nerve transection in rats (18).

Following nerve injury, the Schwann cells in the distal stump upregulate the synthesis of NGF, BDNF, NT-4 and p75 to promote the proliferation and migration of Schwann cells. BDNF also supports motoneuron survival and its level rises along with NT-4 in the denervated distal sciatic nerve stumps. It was also shown that NT-3 supports large myelinating sensory neurons (12). However, the exact roles of these neurotrophins in peripheral nerve regeneration is not clearly defined yet.

Extracellular matrix

The extracellular matrix (ECM) is a naturally occurring substrate, which cells use to migrate, proliferate and differentiate. Four main ECM molecules that are found in the peripheral nervous system and synthesized by Schwann cells are laminin, fibronectin, type IV collagen and various proteoglycans.

Laminin, which is a glycoprotein of cruciform structure comprising three polypeptide chains (19), is found in the Schwann cell basal lamina (20). Laminin, which is known to be the most effective promoter of neurite outgrowth in vitro (19), is also commonly used in tissue culture studies as a substrate to grow explants such as DRGs. Fibronectin is a large highly asymmetrical glycoprotein which is thought to promote adhesion and spreading of cells by linking them to collagen substrates. On the other hand, type IV collagen, which is also a glycoprotein, is found exclusively in the basement membrane of the Schwann cell (21).

Heparan sulphate proteoglycans (HSPG) and chondroitin sulphate proteoglycans (CSPG) and are also found in the basal lamina. HSPGs consist of a polypeptide backbone (core protein) and linear chains of polysaccharides characterized by repeating disaccharides of hexuronate and N-substituted glucosamine residues. The activity of HSPGs as promoters of neurite outgrowth was observed in in vitro studies in the mid eighties. HSPG complexes greatly enhance the neurite outgrowth promoting abilities of laminin or N-CAM (22,23,24). One of the CSPGs found in the peripheral nerve is NG2. NG2 consists of a core protein bearing GAG chains composed entirely of chondroitin sulfate. NG2 levels are upregulated following central nervous system (CNS) injury and the molecule inhibits axon outgrowth (25). Similarly, in vitro studies carried out by me and my colleagues on damaged rodent peripheral nerves showed that NG2 is a significant component of fibroblastic scar tissue and it appears to contribute to the failure of axonal growth through the scar (26). Generally, CSPGs are thought to inhibit axon regeneration and, in contrast, HSPGs promote neurite outgrowth (27).

Other general observations on the neurite promoting activities of heparan sulphate moieties are supported by studies on specific molecules. Perlecan and agrin ECM associated HSPGs, have been associated with the establishment of neuromuscular junctions and CNS axon pathways, respectively (28,29). Cerebroglycan, which is a member of glypican family, is expressed on the surface of cultured neurons and their growth cones, presumably mediating neuronal migration and axon growth (30). Members of the glypican family have also been detected in the developing nervous system and are generally associated with axons rather than glia (31).

Cell surface molecules

Cell surface molecules mediate axonal adhesion and outgrowth, and Schwann cells have been reported to express a variety of these glycoproteins including N-CAM, L1 and myelin-associated glycoprotein.

The distribution of these molecules was described by Martini and Schachner (32) in the regenerating adult mouse sciatic nerve. The authors reported that during the first 26 days post lesion, L1 and N-CAM were detectable at cell contacts between non-myelinating Schwann cells and degenerating axons, and myelin-associated glycoprotein was detectable in the periaxonal area of the degenerating myelinated axons. These authors also observed that growth cones and regenerating axons expressed N-CAM and L1 at contact sites with fibroblast-like cells. Two weeks after nerve transection, when the regenerating axons were

seen in the distal part of the transected nerve, the authors also reported seeing L1- and N-CAM-positive contacts with Schwann cells. Their study clearly shows that L1 and N-CAM are involved in supporting axon regeneration on the surface of Schwann cells and fibroblast-like cells. In addition, studies by Bixby et al. (33) demonstrated 13that other surface molecules such as cadherin, NgCAM (a homophilically binding immunoglobulin-like adhesion molecule) and integrins (which bind laminin, fibronectin and other ECM molecules) are also important in facilitating axon growth on Schwann cells.

Conclusion

Previous and current research data have shown that during peripheral nerve regeneration, Schwann cells, macrophages, mast cells, extracellular matrix molecules and neurotrophins are found to be highly expressed and indeed vital. However, our current understanding of peripheral nerve regeneration is incomplete. Research is still needed to further understand this process, especially in the area of molecular neurobiology, including genomic and proteomic studies. Nevertheless, the available data provide a step towards a better understanding of this complex regenerative process which is useful for better management of nerve injuries.

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ORIGINAL ARTICLE

Attenuation of Hydrogen Peroxide and Ferric Reducing/Antioxidant Power Serum Levels in Colorectal Cancer Patients with Intestinal Parasitic Infection

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Abstract

Background: **This study assessed several common oxidative indices in subjects infected with intestinal parasites, as well as in colorectal cancer (CRC) patients both with and without intestinal parasites.**

Method: **Serum levels of malondialdehyde (MDA), ferric reducing/antioxidant power (FRAP), and hydrogen peroxide (H_2O_2) were measured, as were plasma levels of advanced oxidation protein products (AOPP), all according to established methods. The presence of intestinal parasites was confirmed by stool examination.**

Results: **All intestinal parasiteinfected subjects and CRC patients showed the presence of oxidative stress. Thirtysix percent of the CRC patients had intestinal parasitic infections. The levels of H_2O_2 and FRAP in parasite-infected subjects were significantly higher than in CRC patients, but these levels were significantly lower in the CRC patients with parasitic infections.**

Conclusion: **Parasitic infection and CRC may contribute to oxidative stress independently, but when present together, the oxidative stress burden imposed by parasites may be attenuated.**

Keywords: *Colorectal neoplasm, parasites; oxidative stress, medical sciences*

Introduction

Protozoan and helminth parasites secrete enzymes that generate superoxides and reactive oxygen intermediates (such as hydrogen peroxide (H_2O_2)) in mammalian tissues (1). Reactive oxygen species (ROS) are produced by the imflammatory cells of the host, and these work to kill the invading parasites by nitration, oxidation and chlorination reactions (2). These reactive species have also been implicated as contributing factors in the pathophysiology of cancer (3) and various other diseases (4,5). Oxidative stress occurs as a result of a shift in the balance between the production of free radicals and antioxidant defenses (6) in favour of free radicals, specifically ROS. ROS such as superoxide radicals (O_2), hydroxyl radicals ($OH^{\cdot}$), H_2O_2 and hydroperoxide can inflict oxidative damage on lipids, proteins and nucleic acids (3). This can lead to DNA mutations, which in turn are carcinogenic (7).

The global prevalence of colorectal cancer (CRC) is high, and it accounts for 655,000 deaths out of 58 million deaths worldwide in 2005 (8). In Malaysia, CRC is the most common cancer among men (14.2% of male cancers) and the third most common cancer among women (10.1% of female cancers) (9). Factors such as age, diet, and genetic susceptibility have been suggested to contribute to the aetiology of CRC (10), and these risk factors may exert their effects through prolonged oxidative stress (3). Pathological records and analyses have shown hostand organ dependent correlations between numerous types of cancers and parasitic infections (11,12). However, assessments of the presence of parasites in CRC patients and their effect on oxidative stress are still lacking.

Therefore, in this study, the levels of various oxidative indices in CRC patients and parasite infected patients were compared.

Table 1: Demographic data of the study subjects

Variables	Normal	Subject Groups		
		CRC with parasitic infection	CRC without parasitic infection	Parasitic infection only
n	100	21	38	40
Age	57 ± 12	59 ± 11	57 ± 12	53 ± 15
Gender				
Male	43	12	20	26
Female	57	9	18	14
Chemotherapy Regimen	Nil	Mayo (fluorouracil, foliclinic acid) or Folfox (Folinic acid, Fluorouracil, Oxaliplatin)	Mayo (fluorouracil, folinic acid) or Folfox (Folinic acid, Flouroucacil, Oxaliplatin)	NA

Materials and Methods

Subjects

A total of 59 patients were recruited from the ncology Clinic at the University Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia. All the CRC patients were undergoing chemotherapy. Twenty-one of these patients were confirmed to be infected with one or more intestinal parasites by stool examination and were classified as CRC patients with parasitic infections. Subjects without CRC but positive for parasitic infection were recruited among outpatients attending the UMMC and patients attending medical camps in the Klang Valley, Malaysia. Healthy volunteers (controls, *n*=100) were recruited from the same area where the medical camps were held. The study was approved by the Medical Ethics committee of the UMMC in accordance with the declaration of Helsinki.

Sample collection and preparation

Blood, urine and stool samples were collected from all subjects. Blood samples were collected in plain tubes and EDTA tubes to obtain serum and plasma, respectively. The separated serum and plasma were used for the analysis of oxidative indices. Urine and stool samples were collected in sterile cytology containers. Urinalysis using the dipstick method (Combur Test UX, Roche Diagnostics) was carried out to test for the levels of glucose, bilirubin, nitrite, protein, ketone bodies, urobilinogen and blood. Urine samples that showed biochemical abnormalities were excluded from this study. Parasitological investigation was also carried out on all stool samples to screen for the following parasites: *Entamoeba histolytica, Giardia lamblia, Blastocystis hominis, Microsporidia sp., Dientamoeba fraglis, Ascaris lumbrocoides, Trichuris trichiura,* hookworm and *Taenia sp.* Patients that tested positive for one or more of these parasites were classified as parasite-infected subjects.

Oxidative indices

Serum was used for the assessment of lipid peroxidation (LP), ferric reducing/antioxidant power (FRAP) and H_2O_2, whereas plasma was used for the determination of advanced oxidation protein products (AOPP). LP was determined by measuring malondialdehyde (MDA) according to the modified method of Ratty and Das (12), using 1,1,3,3- tetraethoxypropane as a standard. The FRAP assay was carried out according to the method of Benzie and Strain (13). The reduction of ferric tripyridyltriazine (Fe^{III}TPTZ) complex by the non-enzymatic antioxidants in biological fluids was monitored spectrophotometrically using a kinetic mode at 593nm. Serum H_2O_2 was measured using the ferrous ion oxidative xylenol orange version-2 (FOX-2) method of Benarjee et al. (14). AOPP was measured spectrophotometrically according to the method of WitkoSarsat et al. (15).

Parasitological analysis

Fresh faecal samples were obtained from the subjects and preserved in 10% buffered formalin for parasitological investigation. Intestinal helminths: The formalinethyl acetate concentration (FEAc) method (16) was used to detect *Ascaris lumbrocoides, Trichuris trichuria,* hookworm and *Taenia sp.* Intestinal protozoa, *Dientamoeba fragilis* and *Giardia lamblia* were

Table 2: Non enzymatic oxidant/antioxidant indices in blood samples: Comparison of normal subjects, CRC patients with and without parasitic infections, and subjects with arasitic infections only

Sample Group	n	Oxidative indices			
		AOPP (μmol/L)	FRAP (μmol/L)	H_2O_2 (μmol/L)	MDA (μmol/L)
Normal	100	128.95±4.91	376.56±8.03	20.78±1.05	0.089±0.007
CRC with parasitic infection	21	192.41±85.21 **	370.53±80.15	25.07±3.27 **	0.125±0.071 *
CRC without parasitic infection	38	151.95±49.03 *	358.47±78.16	24.53±4.68 **	0.149±0.081 ***
Parasitic infection only	40	198.63±10.74 *** †	625.01±27.84 *** ## ††	41.90±2.26 *** ## ††	0.173±0.013 ***

Data are given as mean ± SEM
**P<0.001 * P<0.05, **P<0.01, comparison with controls using Student's t-test
Comparison among the diseased groups was analysed using ANOVA
##P<0.001 is the comparison between 'CRC with parasitic infection' and 'Parasitic infection only'
†P<0.01, ††P<0.001 is the comparison between 'CRC without parasitic infection' and 'Parasitic infection only'

screened using the trichrome staining method (17), whereas *Microsporidia sp.* and *Blastocystis hominis* were detected with a modified trichrome staining method (18) and a culture technique (19) respectively.

Statistical analysis

Data were analysed with SPSS for Windows (Version 13.0). All data are expressed as means ± SEM, and significant differences between the patient and control groups were analysed using Student's *t*-test. Comparisons of the measured parameters among the patient groups were analysed using ANOVA.

Results and Discussion

The demographic data of the CRC patients (with and without parasitic infections) and the parasite-infected subjects without CRC are presented in Table 1. All the cancer patients were on chemotherapy, and the one should not rule out the possible influence that different anti-cancer drugs may have on the levels of oxidative damage. However, when we made comparisons among the cancer patients according to the type of chemotherapy they were undergoing, no significant differences were observed (results not shown). The possibility remains that the different chemotherapy protocols affected the oxidative indices, but did so similarly.

Parasite-infected subjects without CRC were also found to be positive for multiple parasitic infections. Comparisons based on the type and multiplicity of the parasitic infection did not show significant differences (result not shown), although this could have been due to the small sample size. Interestingly, 18 CRC patients were positive for *Microsporidia* sp., one was positive for *Blastocystis hominis* and two were positive for both *Microsporidia* sp. and B*lastocystis hominis* (data not shown). In all, 36% (21/59) of the CRC patients tested positive for intestinal parasitic infections, and the most common infection was *Microsporidia* sp. an opportunistic parasite. Rudrapatna et al. (20) reported that only 16.5 % of 1029 cancer patients tested positive for intestinal parasitic infections. Botero et al. (21) showed that in immunocompromised patients, only 9% (10/111) had opportunistic parasitic infections such as *Cryptosporidium* sp., *Microsporidia* sp. and *Strongyloides stercoralis*. Conversely, 42% of Malaysian children with cancer were reported to be positive for intestinal parasites (22).

MDA levels, which are used as an indicator of oxidative stress in cells and tissues (23), reflect the

overall lipid damage level. Lipid damage is a well-known mechanism of cellular injury in humans. We found that serum MDA levels were higher in both CRC patients and parasite-infected subjects (P <0.001 for each) than in normal individuals (Table 2). Numerous studies have shown that MDA levels in both plasma and tumour tissues of CRC patients are elevated (24,25). Also, parasitic infections, especially intestinal parasites, are known to cause cellular damage (26). This could lead to the elevated serum MDA levels in patients with intestinal parasites such as Giardia sp. (27) and Blastocystis hominis (23). However, in the present study, the MDA levels among the three groups were not significantly different (Table 2).

Plasma and serum AOPP levels have been widely used as markers of free radical induced protein damage in renal disease (15,28), diabetic complications (29), patients with parasitic infections and patients with CRC (30,31). To the best of our knowledge, this is the first study that reports elevated plasma AOPP levels in CRC patients with simultaneous parasitic infections. The increased plasma AOPP levels in the CRC and parasite-infected patient groups indicate that these patients have a higher degree of oxidative protein damage than do healthy controls (Table 2). Similarly, Kosova et al. (32) showed that serum AOPP levels were significantly higher in thyroid cancer patients than in normal subjects. AOPP levels in subjects with parasitic infections only were significantly higher than they were in CRC patients without parasitic infection (P <0.01), but were comparable to CRC patients with parasitic infections (Table 2). An in vitro study of nematode parasites demonstrated the existence of an inflammatory system capable of generating hypohalous acids (33) such as HOCl. This could explain the enhanced formation of AOPP in the subjects infected with parasites.

H_2O_2 is an oxidizing agent that can easily be converted into $OH^{\cdot}$ when exposed to ultraviolet rays or ferrous ions (34). Urinary H_2O_2 has been used as an oxidative stress biomarker in malignancy (14) and urinary tract infections (35). The CRC patients and the parasite-infected subjects showed higher levels of H_2O_2 than the healthy controls (Table 2). Interestingly, the H_2O_2 levels in parasite-infected subjects were almost two-fold higher than in CRC patients, but were attenuated in parasite-infected subjects with CRC (Table 2). One possibility is that, in order to fight parasites, monocytes produce $O_2^{\cdot}$ by stimulating xanthine oxidase activity ($O_2^{\cdot}$ is formed when xanthine oxidase converts hypoxanthine to xanthine). This excess $O_2^{\cdot}$ is then converted to H_2O_2 by superoxide dismutase (SOD) (34). When a parasitic infection is present in CRC patients who are on chemotherapy, glutathione peroxidase/catalase (H_2O_2-inactivating enzymes) may be induced, leading to a net reduction in H_2O_2 levels. Chemotherapeutic drugs are generally cytotoxic, and mononuclear cells have been reported to respond to cytotoxicity by increasing glutathione peroxidase levels (36).

FRAP levels are indicative of the total amount of non-enzymatic antioxidants. These include lipid-soluble antioxidants such as vitamin E, vitamin A, and provitamin A (beta-carotene); and water-soluble antioxidants such as vitamin C, uric acid, bilirubin and glutathione. We found that FRAP levels were lower in CRC patients than in healthy subjects (Table 2), possibly due to sequestration by tumour cells and/or oxidants released by the chemotherapeutic agents. Saygili et al. (24) observed reduced plasma vitamin C levels in CRC patients. Interestingly, we found that FRAP levels in the subjects with parasitic infections only were 66% higher than in any of other patients groups (Table 2). At first glance, this result appears to be in conflict with the general notion that under oxidative stress, increased macromolecular damage is accompanied by a reduction in antioxidant levels (37). However, the present findings are supported by another study that demonstrated a significant increase in non-enzymatic antioxidants in the serum of mice infected with *Trichinella spiralis* (38). This increase in FRAP levels in the parasite-infected subjects might be caused by an increase in serum uric acid due to biochemical changes in the body that occur in response to the infection. In agreement with this hypothesis, plasma uric acid levels were found to be elevated in mice with late stage *Plasmodium vinckei* infections (39). Previous studies have reported that increased plasma uric acid may account for as much as 60% of FRAP activity (13), and uric acid may be the main antioxidant in birds (40).

The reduced FRAP level in CRC patients with parasitic infections could be a consequence of the regulation of xanthine oxidase (XO) activity. When a parasitic infection occurs, XO (the dehydrogenase form) converts xanthine (which is generated during superoxide radical production for parasite eradication) to uric acid (41). Since the CRC patients with parasitic infections were also on chemotherapy, XO may be inactivated or inhibited by the drugs, leading to reduced net uric acid production, which in turn is reflected in the attenated FRAP level. A previous study has shown that anti-cancer drugs are potent xanthine oxidase inhibitors (42).

In conclusion, this study provides evidence

that patients with both CRC and parasitic infections secrete significant amounts of serum and plasma metabolites derived from oxidative stress damage. The incidence of parasitic infections in CRC patients is high. Parasitic infection and CRC may contribute to oxidative stress independently, but when present together, the oxidative stress burden imposed by the parasites may be attenuated.

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ORIGINAL ARTICLE

Body Mass Index and Body Fat Status of Men Involved in Sports, Exercise, and Sedentary Activites

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Abstract

A cross-sectional study was carried out in Kota Bharu on three groups of men with ages ranging from 18 to 44 years. The study groups included 83 athletes representing various types of sports and levels of participation (athlete group), 80 active men who exercised a minimum of 30 minutes per day at least 3 times per week (exercise group), and 80 inactive men (sedentary group). The objectives of the study were to compare the body mass indices (BMIs) and body fat statuses among the three groups with different physical activity levels. The height and weight of respondents were measured using the Seca weighing balance with height attachment. Skinfold thickness of biceps, triceps, subscapular regions, and suprailiac regions of each respondent were measured using Harpenden skinfold calipers. Percentage body fat was calculated as the sum of the four measurements of skinfold thickness. The results showed that the mean (± SD) BMIs in the athlete, exercise, and sedentary groups were 22.6 ± 2.9, 23.4 ± 3.5, and 24.3 ± 4.6 kg/m2, respectively. The combined prevalence of pre-obese (BMI 25.029.9) and obese (BMI ³ 30.0) subjects was 21.7% in the athlete group, 29.9% in the exercise group, and 47.5% in the sedentary group. The mean (± SD) percentage of body fat in athletes was 15.7 ± 5.4%, which was lower than in the exercise (18.9 ± 5.5%) and sedentary (20.6 ± 5.8%) groups. The study revealed that individuals who are actively involved in physical activity, particularly in sport activities, have lower BMIs and percentage body fat values compared to sedentary people. Therefore, to prevent obesity, all individuals are encouraged to perform regular physical activity, particularly sports activities.

Keywords: *body fat, body mass index, obesity, physical activity, medical sciences*

Introduction

Obesity can be defined as an excessive accumulation of adipose tissue or fat in the body (1). The basic cause of obesity in most people is that they consume more calories than they expend, and the surplus of food energy is converted into body fat (2). Persons with excess body fat have an increased risk of developing a number of chronic diseases, including cardiovascular diseases, diabetes mellitus, gallbladder disease, osteoarthritis, gout, abnormalities of pulmonary function, and cancer (3). Excess body fat also has significant detrimental effects on physical performance (4). Therefore, measurements of the total body fat of individuals provide useful information. Although the benefits of regular physical activity on health status have been widely recognised, nutritional studies among physically active groups in Malaysia are still lacking. The objective of this study is to compare the body mass indices and body fat contents of athletes, individuals with a moderate level of exercise, and sedentary individuals.

Materials and Methods

This study was carried out among 243 male respondents, aged 18 to 44 years. The respondents studied included 83 sportsmen from eight different types of sports who had participated in various levels of competition (athlete group), 80 active men who had regularly exercised for a minimum of 30 23minutes per day at least 3 times per week during the previous 3 months (exercise group),

and 80 inactive men who reported no physical exercise during the previous 3 months (sedentary group). The athletes were randomly selected from the Kelantan State Sports Council, and several sports associations in Kota Bharu. They were actively involved in sports training with a minimum of 30 minutes per day at least 3 times per week. The respondents in the exercise and sedentary groups were randomly selected from several government departments in Kota Bharu. None of the respondents had a history of chronic diseases.

The height and body weight of the respondents (bare-footed and in light clothing) were measured to the nearest 0.5 cm and 0.1 kg, respectively, using a Seca weighing balance with height attachment. The body mass index (BMI) of each respondent was calculated by dividing body weight in kilograms by height in metres squared (kg/m^2). Body weight classification of respondents based on BMI was determined as described by WHO (5). Waist and hip circumferences were measured using a non-stretchable tape to the nearest 0.1 mm. Waist circumference was measured at the mid-point between the iliac crest and the lower rib margin, while hip measurement was taken as the maximum circumference around the buttocks posteriorly and pubic symphysis anteriorly. The waist-to-hip ratio (WHR) was calculated for each respondent. A WHR cut-off point of greater than 0.9 as an indication of central obesity was used, as recommended by Bray (6).

Skinfold thickness of biceps, triceps, subscapular and suprailiac regions of the respondents were measured using Harpenden Calipers (British Indicators, UK), as recommended by Durnin and Rahaman (7). All the measurements were taken on the left side of the body. Fat content as a percentage of body weight was calculated from the sum of the 4 measurements of skinfold thickness (8). The body fat classification of the respondents was determined as describe by Garrow (9). Lean body mass (LBM) and fat mass (FM) were also calculated for each respondent.

Statistical analysis was done using SPSS version 9.0. Analysis of Covariance (ANCOVA)

Table 1: Age and physical characteristics of the respondents (mean ± SD)

Variables	Respondents		
	Athletes (n = 83)	Exercise (n = 80)	Sedentary (n = 80)
Age (yr)	28.4 ± 6.8	29.4 ± 6.9	29.9 ± 7.2
Weight (kg)	64.2 ± 9.7	65.4 ± 11.1	66.2 ± 14.1
Height (cm)	168.6 ± 5.4 [a]	167.1 ± 5.9 [a]	165.0 ± 5.1 [b]
BMI (kg/m^2)	22.6 ± 2.9 [a]	23.4 ± 3.5 [ab]	24.3 ± 4.6 [b]

BMI = body mass index
[a,b] For each row, different superscript letters indicate significant differences between groups (P < 0.05, ANCOVA)

Table 3: Waist-to hip ratio (WHR) of the respondents (mean ± SD)

Variables	Respondents		
	Athletes (n = 83)	Exercise (n = 80)	Sedentary (n = 80)
Waist circumference	77.06 9.09 a	80.71 ± 9.28 ab	83.15 ± 11.90 b
Hip circumference	93.18 5.73 a	94.42 ± 6.32 a	95.21 ± 8.40 a
WHR	0.82 ± 0.06 a	0.85 ± 0.05 b	0.87 ± 0.06 b
WHR status:			
Desirable (WHR £ 0.90) 55 (68.7)		75 (90.4)	65 (81.3)
High risk# (WHR > 0.90) 25 (31.3)		8 (9.6)	15 (18.7)

a,b For each row, different superscript letters indicate significant differences between groups (P < 0.05, ANCOVA)
Risk of diabetes and cardiovascular disease (6)Values in parentheses denote percentages of respondents

was used to determine differences in mean values between all groups. All values are expressed as mean ± SD. This study was approved by the Research and Ethics Committee, Universiti Sains Malaysia (USM).

Results

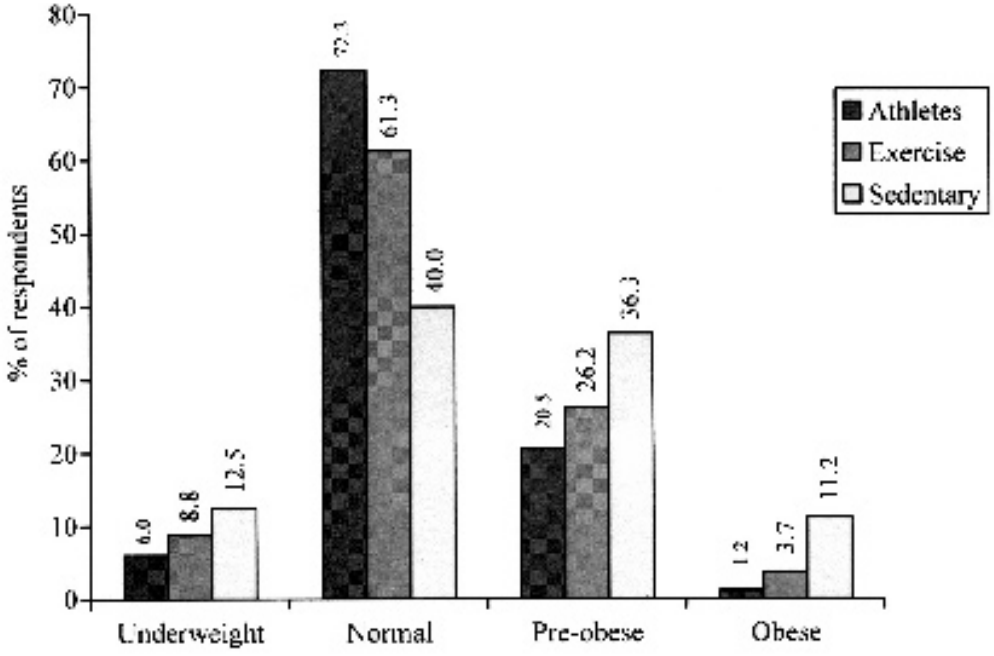
Table 1 shows the mean age and physical characteristics of the respondents. The mean age and body weight were not significantly different between the three respondent groups. The athlete and the exercise groups were significantly taller than the sedentary group. The mean BMIs for the athlete, exercise and sedentary groups were 22.6 ± 2.9 kg/m², 23.4 ± 3.5 kg/m² and 24.3 ± 4.6 kg/m², respectively. The mean BMI was not significantly different between athletes and exercise groups. However, both of the active groups had significantly lower BMIs compared to the sedentary group. The BMI classification according to WHO (5) is shown in Figure 1. The combined prevalence of pre-obese (BMI 25.029.9 kg/m²) and obese (BMI 30.0 kg/m2) individuals was 21.7% in the athlete group, which was lower than in the exercise group (29.9%) and the sedentary group (47.5%).

The mean waist-to-hip ratios (WHRs) among the respondents are shown in Table 3. The athletes had significantly lower mean WHR compared to others. Incidence of WHR above 0.9 (indicative of central obesity) was 9.6% in athletes, which was lower than in the exercise (18.7%) and sedentary (31.3%) groups. The mean skinfold thickness of biceps, triceps, subscapular, and suprailiac regions of the respondents are presented in Table 4.. The athlete group had the lowest mean skinfold thickness compared to the other groups. Thus, the athlete group had the lowest mean fat mass and percentage body fat as compared to the others (Table 5). The mean percentage body fats of the athlete, exercise and sedentary groups were 15.7 ± 5.4%, 18.9 ± 5.5% and 20.6 ± 5.8%, respectively. The incidence of "high fat" among the respondents in this study was determined according to Garrow (9) (percentage body fat more than 22.0%). The incidence was lowest in the athletes (13.3%), compared to almost half (46.2%) of the sedentary group and 32.5% of the exercise group. The mean LBM was not significantly 25different between the three groups (Table 5).

Discussion

The results of this study showed that the athlete group had the lowest mean BMI and percentage body fat compared to the exercise and sedentary groups. The exercise group also had a lower mean BMI compared to the sedentary group. The results

Figure 1: Classification of the body mass index of the respondents*



Note:
* Classification based on WHO (5) : Underweight (BMI <18.5 kg/m²); normal (18.5 24.9kg/m²); Pre-obese (BMI 25.0-29.9 kg/m²); Obese (BMI³ 30.0 kg/m²)

Table 4: Skinfold thickness (mm) of the biceps, triceps, subscapular, and suprailiac regions of the respondents (mean ± SD)

Site of skinfold	Respondents		
	Athletes (n = 83)	Exercise (n = 80)	Sedentary (n = 80)
Biceps (mm)	4.41 ± 1.44 [a]	5.33 ± 2.16 [b]	6.08 ± 2.34 [b]
Triceps (mm)	8.63 ± 3.43 [a]	9.97 ± 3.87 [a]	12.03 ± 4.84 [b]
Subscapular (mm)	13.29 ± 5.28 [a]	16.16 ± 6.50 [b]	18.64 ± 8.90 [b]
Suprailiac (mm)	10.14 ± 5.58 [a]	13.51 ± 6.62 [b]	14.42 ± 6.17 [ba]

[ba,b] For each row, different superscript letters indicate significant differences between groups (P < 0.05, ANCOVA)

Table 5: Lean body mass, fat mass, and percentage of body fat of the respondents (mean ± SD)

Site of skinfold	Respondents		
	Athletes (n = 83)	Exercise (n = 80)	Sedentary (n = 80)
Lean body mass (kg)	53.8 ± 6.7 a	52.6 ± 6.3 a	52.0 ± 8.2 a
Fat mass (kg)	10.4 ± 4.7a	12.9 ± 5.5 b	14.3 ± 6.6 b
Percentage body fat (%)	15.7 ± 5.4a	18.9 ± 5.5 b	20.6 ± 5.8 b
Classification of body fat* [n (%)]:			
Low fat (BF <12.0%)	24 (28.9)	13 (16.3)	8 (10.0)
Average (BF 12.0-22.0%)	48 (57.8)	41 (51.2)	35 (43.8)
High fat (BF >22.0%)	11 (13.3)	26 (32.5)	37 (46.2)

[a,b] For each row, different superscript letters indicate significant differences between groups (P < 0.05, ANCOVA)
* classification based on Garrow (9)
BF = percentage of body fat

can be explained by the difference in physical activity level among the groups. We found that on training days, the mean time spent by the athlete group for physical exercise or sports training was 124 minutes per day, while for the exercise group it was 67 minutes per day. The results are similar to other studies, which have reported that higher activity levels are associated with lower BMI and total body fat (10,11).

The combined prevalence of pre-obese (BMI 25.029.9 kg/m2) and obese (BMI ³ 30.0 kg/m²) individuals in the study groups was the lowest in athletes (21.7%) compared to the other groups. The combined prevalence of pre-obese and obese among the athlete group in this study was also lower than the reported figures of 24% of the general male population in a rural area in Malaysia (12), and 29.5 to 45.0% of the general population in urban areas in Malaysia (1316). However, the combined prevalence of pre-obese and obese individuals in the athlete group was higher compared to the 12% of 84 male national athletes reported by Wan Nudri et al. (17). Yet, it is interesting to note that the mean BMI of the athlete group in this study (22.6 ± 2.9 kg/m²) was similar to that of the national athletes (22.9 3.5 kg/m²) reported earlier (17).

Based on BMI, the prevalence of pre-obese and obese individuals in the active groups could be considered to be rather high (21.7% of the athletes and 29.9% of the exercise group). However, it is important to note that BMI has limitations in classifying obesity among active individuals, because BMI does not distinguish between weight associated with fat and weight associated with muscle (17,18). For the present study, this situation was true for the athlete group but not for the exercise group. Based on percentage body fat (9), the prevalence of "high fat" (body fat > 22.0%) or "really pre-obese or obese" among the athlete group was only 13.3%, while among the exercise group it was 32.5%.

The athletes had significantly lower mean WHR compared to others. WHR provides an index of regional fat distribution and has proven valuable as a guide to health risk. According to Bray (6), men who have WHR values above 0.9

have higher risks of mortality due to diabetes and cardiovascular diseases. Fat distribution is a more important risk factor for morbidity and mortality than being overweight per se and has a relative risk ratio of ³ 2 (6). WHR measurements of adult populations have not been studied extensively in Malaysia. The available data show that the mean WHR of the general population ranges from 0.84 to 0.91 (12,19,20), which is higher than the mean WHR (0.82) of the athletes in the present study.

The athlete group had the lowest mean percentage body fat compared to the other groups. However, the mean percentage body fat of the athlete group (15.7 ± 5.4%) in this study was slightly higher compared to the 13.8 ± 4.5% value of national athletes (17). A study in Nigeria among national athletes and the general population reported that the mean percentage body fat among the athletes was 15.8 0.02%, which was lower compared to non-athletes of 18.1 ± 0.05% (21). A study in Malaysia of the general population by Chee et al. (22) on 117 male adults aged 1958 years reported that the mean percentage body fat was 21.0 ± 4.7%. Ismail et al. reported that the mean percentage body fat among 20 Malaysian male soldiers, a relatively physically active group, was 16.6 ± 3.4% (23).

The results from the present study, together with findings from other studies locally and in other countries reveal that individuals who are actively involved in physical activities, particularly sports activities, have better health statuses compared to less active groups, as shown by the lower BMIs and percentage body fats. Physical activity is associated with protective effects and decreased risk of cardiovascular disease and cardiovascular disease mortality. A study of Harvard alumni indicated that adopting a physically active lifestyle involving walking, climbing stairs, and playing sports delays all-cause mortality and extends longevity up to 1.6 years (24). Sedentary alumni who expended less than 1,500 kcal/week were at a 39% higher risk of early death than their more active counterparts (24). Therefore, all individuals are recommended to perform regular physical activities. According to public health recommendations, all individuals should accumulate a minimum of 30 minutes of moderate physical activity on most and preferably all days of the week (25).

Conclusions

Physically active groups, especially athletes had BMIs and percentage body fats compared to less active groups. Therefore, besides maintaining a healthy diet, all individuals are encouraged to participate in sports or other physical activities for a minimum of 30 minutes per day at least 3 times per week to avoid the emergence of chronic diseases.

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Conception and design: WAMWM, WNWDStatistical expertise: MRAAll authors have contributed equally to the analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, and final approval of the manuscript.

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ORIGINAL ARTICLE

Antimicrobial susceptibility of clinical isolates of *Pseudomonas aeruginosa* from a Malaysian Hospital

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Abstract

Ongoing surveillance of Pseudomonas aeruginosa resistance against antimicrobial agents is fundamental to monitor trends in susceptibility patterns and to appropriately guide clinicians in choosing empirical or directed therapy. The in vitro activity level of eight antimicrobial drugs was assessed against 97 clinical isolates of *P. aeruginosa* collected consecutively for three months in 2007 from a Malaysian hospital. Antimicrobial susceptibility was determined using the E-test method in addition to the hospital's routine diagnostic testing by the disk diffusion method. Respiratory and wound swab isolates were the most frequently encountered isolates. The E-test and disk diffusion methods showed high concordance in determining the in vitro activity of the antimicrobial agents against the *E* isolates. Piperacillin-tazobactam was the most active antimicrobial agent with 91.8% susceptibility, followed by the aminoglycosides (amikacin, 86.6% and gentamicin, 84.5%), the quinolone (ciprofloxacin, 83.5%) and the beta-lactams (cefepime, 80.4%, ceftazidime, 80.4%, imipenem, 79.4% and meropenem, 77.3%). Incidence of multidrug resistance was 19.6% (19 out of 97 isolates). Periodic antibiotic resistance surveillance is fundamental to monitor changes in susceptibility patterns in a hospital setting.

Keywords: *antibacterial agents, bacterial drug resistance, Pseudomonas aeruginosa, medical sciences*

Introduction

Pseudomonas aeruginosa is an aerobic, motile, nutritionally versatile, gram-negative rod exhibiting intrinsic resistance to several antimicrobial agents (1,2). The rapid increase of drug resistance in clinical isolates of this opportunistic human pathogen is of worldwide concern (3,4,5,6,7).

Ongoing surveillance of *P. aeruginosa* resistance against antimicrobial agents is fundamental to monitor trends in susceptibility patterns and to appropriately guide the clinician in choosing empirical or directed therapy, especially when new antimicrobial agents may not be readily available in the near future (8,9). However, there are few recent surveillance studies reporting antimicrobial resistance patterns of *P. aeruginosa* in Malaysia (10,11). Thus, in this study, we assessed the current in vitro activity level of eight antimicrobial drugs against clinical isolates of *P. aeruginosa* obtained from the Kuala Lumpur Hospital. The concordance between the E-test and disk diffusion aeruginosamethods in antimicrobial susceptibility testing was also evaluated.

Materials and Methods

Clinical isolates

A total of 97 consecutive clinical isolates of *P. aeruginosa* were collected between October 2007 and December 2007 at the Kuala Lumpur Hospital, Malaysia a government tertiary referral hospital with 81 wards and 2,502 beds. Of the 97 specimens, 21 were obtained from general paediatric wards, 20 from general medicine wards, 14 from neurology wards, 11 from intensive care units, 9 from orthopaedic wards, 7 from general surgery wards, 5 from respiratory medicine, 4 from urology wards, 2 from uronephrology and 1 each from dermatology,

ENT (ear, nose and throat), burn and nephrology wards. The isolates were identified by standard laboratory methods (1).

Antibiotic susceptibility test

Minimal inhibitory concentrations (MICs) of piperacillin-tazobactam, ceftazidime, cefepime, imipenem, meropenem, gentamicin, amikacin and ciprofloxacin were determined by E-test (AB Biodisk, Solna, Sweden) in addition to the hospital's routine antimicrobial susceptibility testing by the disk diffusion method. Results of E-test and disk diffusion methods were interpreted in accordance to the Clinical and Laboratory Standards Institute (CLSI) (12). Control strains included *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922.

Multidrug-resistant (MDR) isolates were defined as isolates demonstrating resistance to antimicrobials from at least two of the five antipseudomonal classes of antimicrobial drugs tested in this study: piperacillin-tazobactam, cephalosporins, carbapenems, aminoglycosides and fluoroquinolones.

Statistical Analysis

Statistical analysis was done using SPSS software, version 15. Statistical analysis by Spearman's rank correlation was carried out to assess the correlation in susceptibility between two drugs. Cross-tab analysis was performed to obtain a Kappa value to measure the concordance between E-test and disk diffusion methods. The percent concordance of the two methods was calculated as follows: $[(a + d)/(a + b + c + d)]*100$, where *a* is the number of isolates sensitive by both tests, *b* is the number of isolates sensitive by E-test and resistant by disk diffusion, *c* is the number of isolates resistant by E-test and sensitive by disk diffusion, and *d* is the number of isolates resistant by both tests (13). The Spearman's rank correlation was also performed to evaluate the association between occurrence of drug resistance and i) ward of patient origin and ii) specimen of isolates. In all cases, a *P* value of < 0.05 was considered indicative of significance.

Results

The results of the antimicrobial susceptibility testing are shown in Table 1. Piperacillin-tazobactam was the most active antimicrobial agent in vitro with 91.8% susceptibility, followed by the aminoglycosides (amikacin and gentamicin), quinolone (ciprofloxacin), the cephalosporins (ceftazidime and cefepime) and the carbapenems (meropenem and imipenem).

Twenty-five isolates were resistant to at least one of the five antipseudomonal classes of antimicrobial agents and revealed a total of 12 antimicrobial resistance patterns (Table 2). The most prevalent pattern, P2, displaying resistance to all antimicrobial drugs except piperacillin-tazobactam was observed in 9 (36%) of the 25 isolates. The MIC of piperacillin-tazobactam on these isolates was between 3 and 16 ìg/mL. Pattern P7 was the second most common with resistance to piperacillin-tazobactam, the cephalosporins and the carbapenems. Pattern P9 exhibited resistance to the carbapenems in 3 isolates. Two isolates were resistant to all antimicrobial agents tested. Resistance to both carbapenems was observed in 20 of the 25 isolates. The overall incidence

Table 1: Antimicrobial susceptibility of *P. aeruginosa* isolates to eight antimicrobial agent

Antimicrobial agent	% susceptible	MIC (µg/mL)			No. of isolates [MIC (µg/mL) breakpoint]		
		50%	90%	Range	S	I	R
PT	92.8	4	24	1 - >256	90 [<64]	0	7 [>128]
CAZ	80.4	2	>256	0.5 - >256	78 [<8]	0	19 [>32]
CPE	80.4	2	>256	0.09 - >256	78 [<8]	0	19 [>32]
IMP	79.4	1	>32	0.25 - >32	77 [<4]	1	19 [>16]
MER	77.3	0.25	>32	0.032 - >32	75 [<4]	1	21 [>16]
GN	84.5	3	96	1 - >256	82 [<4]	0	15 [>8]
AK	86.6	4	32	2 - >256	84 [<16]	4	9 [>32]
CIP	83.5	0.19	>32	0.064 - >32	81 [<1]	1	15 [>4]

Note: S, sensitive; I, intermediate; R, resistant
PT=piperacilin-tazobactam, CAZ=ceftazidime, CPE=cefepime, IMP=imipenem, MER=meropenem, GN=gentamicin, AK=amikacin, CIP=ciprofloxacin

Table 2: Antibiotype patterns of the P. aeruginosa strains exhibiting resistance to at least one antimicrobial agent

Pattern type	Antimicrobial class*								Number of strains with pattern (%)
	PT	Cephalosporin		Carbapenem		Aminoglycoside		Quinolone	
	PT	CAZ	CPE	IMP	MER	GN	AK	CIP	
P1	R	R	R	R	R	R	R	R	2 (8)
P2		R	R	R	R	R	R	R	9 (36)
P3	R		R	R	R	R		R	1 (4)
P4		R	R	R	R	R		R	1 (4)
P5		R	R		R	R	R	R	1 (4)
P6		R		R	R	R	R	R	1 (4)
P7	R	R	R	R	R				3 (12)
P8	R	R	R						1 (4)
P9		R	R						1 (4)
P10				R	R				3 (12)
P11					R				1 (4)
P12								R	1 (4)
Total									25 (100)

Note: Isolates above broken lines are MDR
*See Footnote Table 1
R=resistant

of multidrug resistance was 19.6% (19 out of 97 isolates).

Table 3 shows the distribution of the 97 *P.* aeruginosa isolates according to the specimen type and its correlation to multidrug resistance. The E-test and disk diffusion methods showed high percentage of concordance (>96%) and an excellent Kappa measure of agreement (0.8 to 1) (Table 4).

Discussion

Periodic antimicrobial resistance monitoring in *P. aeruginosa* is fundamental to updating the current activity level of commonly used antipseudomonal drugs. In the present study, the carbapenems were the least active agents evaluated with only 77.3% and 79.4% of isolates being susceptible to meropenem and imipenem, respectively. Imipenem has been reported to be very active against *P. aeruginosa* in a number of recent studies, (3,10,14) while others have reported otherwise (6,15). A study done in another tertiary care hospital in Malaysia (10) involving isolates collected in 2005 reported a low incidence of imipenem resistance (9.90%) compared to the present (20.6%). Another Malaysia/Singapore study in 1999 that did not include our hospital found imipenem to be the most active â-lactam (14.7% resistance), but cefepime and piperacillin-tazobactam had higher resistance rates than the 31present study (11). Varying drug resistance levels in different hospitals in the same country have been reported in the past and is attributed to the differential usage of antibiotics in the respective hospitals. An Indian study (4) noted that the low incidence of imipenem resistance (7.2%) at their hospital compared to a higher resistance rate detected in another setting in the same country (16) was due to the fact that imipenem is still used as a reserve drug in the former. In general, when compared to previous Malaysian studies (10,11), our study showed higher resistance rates to all drugs tested except cefepime, meropenem and piperacillin-tazobactam. However, the difference in MDR rates between the present and other studies could not be compared due to varying definitions of multidrug resistance.

A number of studies found piperacillin-tazobactam to be either the most active antimicrobial agent against *P. aeruginosa* or the second most active after amikacin (3, 4,7,10,17). However, a recent report has questioned the appropriateness of the current CLSI resistance breakpoint of piperacillin-tazobactam since the study discovered an increased mortality rate

Table 3: Distribution of the P. aeruginosa isolates according to the specimen type and its correlation to multidrug resistance

Type of specimen	No (%) isolates studied (n=97)	No MDR isolates (n=19)	Spearma's rho[a]
respiratory tract	40 (41.2)	11	0.167
wound swab	52 (33.0)	1	-0.291[b]
urine	15 (15.5)	5	0.148
blood	5 (5.2)	2	0.120
tissue	4 (4.1)	0	-0.102
CSF	1 (1.0)	0	-0.50

n= total number
[a]Values are Spearman correlation coefficient. The sign of the correlation coefficient indicates the direction of the relationship (positive or negative)
[b]Highly signifivant correlation (*P*<0.001)

Table 3: Agreement between E-test and disk diffusion methods

Antimicrobial agent	% agreement	Measure of agreement, Kappa[a]
Piperacillin-tazobactam	98	0.823
Ceftazidime	99	0.967
Cefefime	97	0.896
Imipenem	98	0.937
Meropenem	98	0.939
Gentamicin	99	0.959
Amikacin	98	0.905
Cirofloxacin	99	0.962

[a]poor agreement = <0.20; fair agreement = 0.200.40; moderate agreement = 0.400.60; good agreement = 0.600.80; very good agreement = 0.801.00

associated with empiric piperacillin-tazobactam therapy given to patients with *P. aeruginosa* bacteraemia; the isolates had reduced piperacillin-tazobactam susceptibility (18).

Although amikacin was the second most potent drug in vitro, the resistance rate was higher compared to other studies (5,6,7,10). In the other studies, the resistance rate of amikacin was far lower than its aminoglycoside counterpart, gentamicin. In the present study, however, there was a significant correlation between the two aminoglycosides (rho > 0.9, $P < 0.01$), although the MIC_{90} value of amikacin (32) was lower than that of gentamicin (96). A significant correlation between class members was also observed among the cephalosporins and carbapenems (rho > 0.9, $P < 0.01$) with equal MIC_{90} values (i.e., > 250 and > 32, respectively).

The high percentage of concordance and an excellent Kappa measure of agreement showed that both methods have high agreement in determining the in vitro activity of the antimicrobial agents on *P. aeruginosa* isolates, which corroborates similar studies (19,20) that reported an excellent and acceptable correlation, respectively, between the disk diffusion and E-test methods. Therefore, although the E-test is rapid, easy to perform and has an added ability to determine MIC value, the disk diffusion method is equally reliable and more cost-effective for routine hospital use.

There was no significant correlation between drug resistance and the wards from which isolates originated (data not shown). The distribution rank of the isolates according to the types of specimens (respiratory > wound swab > urine > blood) was similar to that described by a worldwide SENTRY antimicrobial surveillance study (8), even though the total number of isolates included in the present study is incomparably small. Respiratory isolates (41.2%), including tracheal and nasopharyngeal aspirates as well as sputum, were the most frequently encountered. *P. aeruginosa* isolates

from respiratory tract also showed the highest rate of multidrug resistance, as observed in a similar study of inpatient isolates done in a Saudi Arabian hospital (21). Wound swab isolates (33.0%) were the second most frequently encountered. However, the incidence of resistance was statistically less likely to be observed in these isolates ($P < 0.001$). Of the 32 pus isolates, 31 were fully susceptible to all the antimicrobial agents tested, suggesting that wound swab isolates are less likely to be multidrug resistant. Nevertheless, the correlation between specimen type and multidrug resistance would have been more noteworthy if supported by data on patients' clinical conditions, which is a limitation of our study.

In conclusion, the higher resistance rate, when compared to previous studies, calls for prudent use of antibiotics in order to limit further increases in resistance. Antimicrobial surveillance should be done periodically to monitor the current susceptibility patterns in local hospitals. A standard definition of P. aeruginosa multidrug resistance will allow better comparisons between studies.

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CASE REPORT

Theophylline toxicity: A case report of the survival of an undiagnosed patient who presented to the emergency department

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Abstract

Theophylline toxicity is a life-threatening toxidrome that can present to an emergency department. To ascertain an immediate provisional diagnosis in toxicology at the emergency department is very challenging, especially when the patient presents with altered mental status, because the clinical features of several toxidromes overlap. We report a case of survival of undiagnosed theophylline toxicity that required intubation for two days in the intensive care unit. This was the first case to have been reported from our department. Accurate diagnosis of a toxidrome by gaining adequate history and conducting a thorough physical examination and early serum toxicology screening, coupled with good knowledge of toxicology, will lead to better patient outcomes.

Keywords: *Theophylline, toxicity, emergency medicine, medical sciences*

Introduction

Theophylline is a commonly used drug in the treatment of acute or chronic lung diseases. Despite the considerable potential benefit of theophylline, its narrow therapeutic index and erratic absorption and elimination contribute to the potential for toxicity, which can have high morbidity and mortality (1,2). The toxidrome manifestation can present as an overlap with other drug toxicities, especially in unmonitored patients who have altered higher mental functions.We present a case of undiagnosed theophylline toxicity due to attempted suicide with sustained generalised tonic-clonic seizures with altered mental status and supraventricular tachycardia. As this was our first experience encountering such a case, we recommend a few measures that can be taken when managing a patient with an undiagnosed drug toxicity or toxidrome in the emergency department.

Case report

A 22-year-old man was brought to the Emergency Department (ED) of Universiti Sains Malaysia Hospital (HUSM) by family members 10 hours after the suspected ingestion 30 tablets of chlorpheniramine. He developed intermittent nausea and non-projectile vomiting (containing food particles) with abdominal pain 2 hours post-ingestion, and the symptoms persisted until he presented to the ED. On arrival, he appeared drowsy, but was not in respiratory distress. His pulse was persistently tachycardic to more than 120 beats/minute with a regular rhythm, and he was hypotensive, with a blood pressure of 90/64 mmHg. His axillary temperature was 37.0ÚC with moist skin. His pupils were 3 mm bilaterally, equal, and reactive to light. Physical examinations of other systems were unremarkable. His electrocardiogram showed sinus tachycardia, and his capillary blood sugar was 6.7 mmol/L. In the ED, he complained of epigastric pain and urinary retention. Intravenous metoclopramide (10 mg) was prescribed to relieve vomiting along with intravenous ranitidine (50 mg), followed by activated charcoal. The patient was still hypotensive despite adequate fluid resuscitation. To restore normal blood pressure, the patient was started on an infusion of noradrenaline, which targets the peripheral alpha-1 receptor. After 2 hours in the ED, the patient underwent two generalised tonic-clonic seizures. Each episode lasted approximately 10 minutes and was aborted with intravenous

diazepam. He was given intravenous phenytoin and was later electively intubated for airway protection and cerebral resuscitation. Initial blood gases showed metabolic alkalosis, and he was hypokalemic (see Table 1).

The patient was admitted to the Intensive Care Unit (ICU) for monitoring and supportive care. While in the ICU, he developed a supraventricular tachycardia (SVT) and synchronised cardioversion (50 J) was delivered, which successfully reverted him back to sinus rhythm. The patient self-extubated on day two of his hospitalisation. His blood pressure was normotensive on inotropic support, but the pulse rate remained tachycardic. Further history elicited from the patient after he regained consciousness revealed that he took 30 tablets of Neulin SR 250 mg. His theophylline level was assessed immediately, and was found to be 40.4 μg/mL at 72 hours post-ingestion (see Table 2). Repeated blood gases persistently showed mild metabolic acidosis, and he also had increased blood urea and creatinine. His creatinine phospokinase (CPK) level was >10,000 IU/L, but urine myoglobin was negative. Intravenous fluid was increased to 150 mL/kg/d, and an intravenous furosemide infusion was started. He was referred to a nephrologist due to the development of acute renal failure, and due to the toxic level of theophylline he was referred for hemoperfusion therapy to enhance theophylline elimination (see Table 3). However, he was treated conservatively. His theophylline level eventually decreased to 20.54 μg/mL and 2.834 μg/ml at hospital day 5 and day 6, respectively. His CPK level decreased to 7471 IU/L, and three repeated urine tests for myoglobin were negative. His blood pressure and heart rate later normalised, and he was transferred out to the High Dependency Unit (HDU) at hospital day 7. The patient was discharged well from the hospital after 2 weeks of hospitalisation without neurological deficit and with normalised renal function.

Discussion

We here describe a young patient who had allegedly intentionally ingested 30 tablets of 'chlorpheniramine,' but who at day 3 of hospitalisation admitted to having taken theophylline SR tablets, and was in acute theophylline toxicity. To ascertain an immediate provisional toxicological diagnosis in the emergency department is very challenging, especially when the patient presents with altered mental status. The clinical features of one toxidrome often overlap with other toxidromes.In this case, the patient initially presented at 10 hours post-ingestion with a history of abdominal pain, urinary retention, nausea, and vomiting, and with normal body temperature, equal pupil sizes, and altered mental status. At the emergency department, he developed hypotension and sinus tachycardia, and he subsequently developed generalised tonic-clonic seizures and supraventricular tachycardia (SVT). The presenting complaints were not a common presentation of anticholinergic toxicity (the incongruous findings were hypotension, moist skin with a normal body temperature, the pupil sizes and hypokalaemia). Such findings were more closely associated with toxicity related to theophylline, tricyclic antidepressants or phenothiazines. In general, the common presentations of chlorpheniramine (anticholinergic) toxicity are dementia with mumbling speech, tachycardia, dry flushed skin, dilated pupils, myoclonus, slightly elevated temperature, urinary retention, decreased bowel sounds, seizures, and cardiac arrhythmias (3).

The best initial emergency department management of the patient depends on an accurate

Table 1: The serial monitoring of arterial blood gases (ABG) of the patient during pre-resuscitation, resuscitation and post-resuscitation

Measurement	Time						
	Immediately after arrival	After Endoctracheal Intubation	2d	3d	4d	5d	6d
pH	7.475	7.188	7.339	7.192	7.44	7.44	7.395
pCO_2 (mmHg)	34.2	27.3	23.1	36.6	22.2	19.2	32.9
pO2 (mmHg)	291	56.5	187	181	195	63.4	165
Base excess	1.6	-16.6	-12.6	-13.1	-8.5	-10.5	-4.9
HCO_3	26.4	12.1	15.1	14.1	18.6	17.0	20.9

Note: d=days

Table 2 : Serial monitoring of theophylline levels after the patient regained consciousness in the intensive care unit, HUSM

Time (hours)	Serum theophylline level (µg/mL)
72	40.4
96	20.5
120	2.83

toxicological diagnosis. Had theophylline toxicity been suspected initially, intermittent gastric lavage with activated charcoal (46 grams every 46 hours) could be instituted aggressively. This would double the rate of elimination of theophylline. Alkaline diuresis could also be considered in this case, but is not generally recommended (4).

The most common metabolic responses to theophylline toxicity in the literature are hypokalaemia, hyperglycaemia, lactic acidosis, hypomagnesaemia and hypophosphataemia (4,5,6). Thus, the presence of such abnormalities should raise suspicion of overt theophylline toxidromes. In this situation, toxic screening would be beneficial. If screening is done early in the emergency department, it can guide the specific management of the drug toxicity.

Gastric emptying before administration of activated charcoal appears not to improve outcomes. There are several studies that failed to document any benefits from gastric emptying prior to the administration of activated charcoal . (7) Gastric lavage should not be considered unless a patient has ingested a potentially life-threatening amount of a poison. The procedure can be performed within 60 minutes of ingestion (8). Even then, a clinical benefit has not been confirmed in any controlled studies, according to the American Academy of Clinical Toxicology/ European Association of Poisons Centres and Clinical Toxicologists Position Statement (9). Therefore, the best method available in the ED to reduce drug absorption from the gastrointestinal tract is the administration of activated charcoal, which is both rapid and very efficient. Orally-given activated charcoal may be the most effective treatment for a sustained-release theophylline overdose. The maximum benefit is obtained when it is administered soon after an overdose, although later administration might still be of value (10). It has been suggested that the mechanism of action of charcoal in theopylline toxicity is the adsorption of theophylline in the lumen of the gut through direct dialysis of theophylline across the gut mucosal capillaries. This interrupts enterohepatic circulation of the drug and promotes back-diffusion of theophylline from the blood to the intestine (11). Multiple doses of activated charcoal (MDAC) are recommended in patients who have ingested a life-threatening amount of theophylline (12). MDAC have been shown to significantly enhance the elimination of both parenterally administered and orally administered theophylline (12,13,14,15). The dosing should be 3040 g every 4 hours, but equal benefit has been demonstrated by giving smaller doses more frequently (16). In a case of potentially severe or life-threatening toxicity, extracorporeal elimination is recommended. The choices are charcoal hemoperfusion, continuous haemofiltration, or haemodialysis (17,18). In cases of slow-release theophylline overdose with rising theophylline levels and patient deterioration despite adequate gastrointestinal decontamination, gastric pharmacobezoar formation (the residue of sustained-release formulations) should be suspected. This can result in fatality (19). There have also been reported cases of successful treatment of theophylline toxicity by upper gastrointestinal endoscopic removal of all the tablets in the stomach 4 hours after ingestion (20).

Intractable vomiting caused by theophylline toxicity can be controlled with a variety of antiemetics such as metoclopromide, prochloperazine and droperidol. Some authors recommend the use of ranitidine in conjunction with an antiemetic, as the reducing xanthine will induce a decrease in gastric acidity and a reduction in gastric acid volume. Ranitidine is preferable to cimetidine because ranitidine interferes less with hepatic clearance (21). However, there are reported cases that ranitidine can induce chronic theophylline toxicity in the elderly (22). Animal studies in rats concluded that cimetidine and ranitidine can induce interstitial nephritis, and thus these drugs should be used with caution. In the described patient, the renal profile on arrival was already deranged, and thus ranitidine was used with caution. Ranitidine has the potential to worsen renal function and delay its own clearance. Thus, its interference with hepatic metabolism can be prolonged (23). This is important to keep in mind when managing patients with theophylline toxicity.

Seizure is a sign of neurotoxicity and is considered a poor prognostic sign. The serum concentration at which seizures are triggered is unknown. Intravenous diazepam may be used alone to control the seizures but it is not very

Table 3 : Serial monitoring of blood urea and serum electrolytes (BUSE) of the patient during pre-resuscitation and post-resuscitation

BUSE (mmol/L)	Time (days)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Urea	8.0	8.0	14.5	18.7	16.3	32.9	32.9	35.4	31.3	31
K	2.6	3.0	4.8	5.7	5.1	5.0	5.0	4.4	4.3	4.0
Na	132	137	145	147	152	154	154	153	148	148
Creat	160	178	307	367	373	603	603	577	530	501

effective in most cases. Phenytoin, phenobarbitone or carbamazepine can be used in combination, because these drugs increase theophylline metabolism, thus reducing the theophylline serum concentration (24). Short-acting antiepileptic use is advocated because they enhance the rate of elimination of theophylline through activation of CYP3A4 (25). They also decrease absorption of theophylline through activation of gut CYP3A4 proteins, which enhances first-pass metabolism for theophylline. Aside from their use as anticonvulsants, antiepileptics can also be useful in drug-induced supraventricular tachycardia.

In this case, extracorporeal elimination of toxin would have been beneficial, but the theophylline toxicity was diagnosed too late. Due to the delay in diagnosis, the patient developed seizures and hypotensive episodes that required inotropic support, a sign of life-threatening intoxication.

In conclusion, an accurate diagnosis of a toxidrome by gaining adequate history and conducting a thorough physical examination and early serum toxicology screening, coupled with good knowledge of toxicology, will lead to better patient outcomes.

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CASE REPORT

Facial Nerve Paralysis: A Rare Complication of Parotid Abscess

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Abstract

Benign parotid neoplasm and inflammatory processes of the parotid resulting in facial paralysis are extremely rare. We report a 72 -year-old Malay female with poorly-controlled *diabetes mellitus* who presented with a painful right parotid swelling associated with right facial nerve palsy. The paralysis (Grade VI, House and Brackmann classification) remained after six months.

Keywords: *facial nerve injuries, parotid neoplasms, parotitis, neurosciences*

Introduction

Facial paralysis associated with benign inflammation in and around the parotid gland is unusual in the absence of a malignant process. The association appears to be rare with less than 15 cases being reported in the literature (15).

Case Report

A 72-year-old Malay female with poorly-controlled *diabetes mellitus* presented with a five-day history of painful swelling of the right parotid, trismus and fever. Three days later, she developed a Grade III right facial nerve palsy that later progressed to Grade VI (House and Brackmann classification). She denied any history of parotid swelling. Examination revealed a diffuse, inflamed and tender swelling of the right parotid gland measuring approximately 5 x 7 cm with purulent discharge noted from the right Stensen's duct. There were no palpable stones within the parotid duct. There was a complete right lower motor neuron facial nerve palsy. The remainder of the examination was unremarkable. A parotid abscess with facial nerve palsy was the final impression.

Complete blood count revealed a total white cell count of 29.6 x 109/L and a blood glucose of 18.6 mmol/L. She was started on intravenous amoxicillin and subcutaneous insulin. A CT scan was not performed, as there was no evidence of parapharyngeal extension.

She underwent incision and drainage of the abscess via a modified Blair incision. Intra-operatively, there was massive necrosis involving the deep lobe of the parotid gland, which mandates aggressive surgical debridement. Identifying the facial nerve was difficult because necrotic tissue had replaced most of the normal parotid tissue. One week after the first operation, she underwent further surgical debridement to remove necrotic parotid tissue. Subsequently, the large wound created by aggressive debridement was closed with a V-Y advancement flap. The tissue biopsy obtained during the first operation showed necrotic tissue with no evidence of malignancy or tuberculosis. Culture of the pus grew Klebsiella spp. that was sensitive to amoxicillin. Grade VI paralysis remained at six months.

Discussion

Parotid abscess is a condition seen mainly in elderly, diabetic, and immunocompromised patients, as presented in this case. Ascending bacterial migration from the oral cavity to the salivary duct is the likely route of bacterial entry. However, this patient did not have any predisposing factors for parotitis (e.g. poor oral hygiene, Stensen's duct obstruction, dehydration).

The incidence and the pathophysiology of facial palsy in conjunction with parotid abscess or parotitis are unknown. It may range from a partial 40to total paralysis. Multiple and poorly-understood factors may account for facial dysfunction in the setting of parotid gland inflammation. Various mechanisms have been proposed to account for the nerve involvement in a non-malignant lesion. These include direct pressure (6), inflammation and necrosis (7) and haemorrhage into a cyst or a tumour (8). In this case and other suppurative diseases, the virulence of the organisms, the extent of perineuritis, and acute nerve compression are likely to be the causes. Nerve damage may be induced by increased pressure over an abbreviated time in addition to the toxic effects of surrounding inflammation (9).

Management of parotitis complicated by facial paralysis should initially be conservative with aggressive broad spectrum antibiotics, rehydration, sialogogues (e.g. lemon juice) and good oral hygiene (9). In most reported cases of parotitis with facial palsy, causative organisms were not identified. Therefore, it is important to use a broad-spectrum antibiotic until a specific organism can be isolated. Most cases report recovery of the facial nerve after resolution of the acute inflammatory process (9). Surgery does not play a major role in the treatment of isolated inflammatory disorders of the parotid gland unless there is abscess formation and facial nerve palsy. The surgical technique involves elevating a standard parotidectomy flap with careful dissection of the facial nerve fibres. After elevation of a skin flap superficial to the parotid fascia, a haemostat is introduced to make multiple openings into the parotid gland and is spread in the direction of the facial nerve branches. An attempt should be made to identify the facial nerve by standard methods or by using facial nerve stimulator. However, with a fulminant necrotizing process, debridement of all areas of necrosis may compromise full return of facial nerve function (4,9) as occurred in this case.

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