The Value of Exercise in Health Promotion:

The Message is Clear, but Who's Listening?

"There is something infinitely sad about citizens cherishing the right to slowly kill themselves" said Minister for Health and Children, Mr Micheál Martin, on the 7th of March, 2000 in the course of a Dáil debate dealing with his proposal to introduce new anti-tobacco legislation. He was responding to claims by certain members of the Vintner's Federation of Ireland that his proposals were draconian and would neither be supported nor adhered to by them. One went so far to describe a smoky Irish pub as a national institution which could not and should not be threatened.

Yet despite all the odds, it appears that by the launch of this Journal the impossible may have actually happened. The smoking ban is due to come into effect on March 29th and at present threats of militant defiance by irate pub owners appear to be subsiding. Increasingly the outrage is being replaced by a gentle acceptance that the ban will come in and that life will go on.

The ban on smoking in the workplace represents not just the courage of one politician, but a major shift in our national approach to health and well-being. For historical and resource reasons our healthcare emphasis has in the past almost exclusively focused on reaction rather than action. We formerly prioritised last minute intervention; "fire- fighting" each crisis as it arose. By contrast, we now seem to be embracing in a meaningful way the merits of disease prevention and health promotion.

This shift in our focus hasn't come before time. Almost half of all premature deaths in developed countries such as Ireland are caused by lifestyle related problems. Changes in physical activity, diet and lifestyle following rapid globalisation and urbanisation pose increasing challenges. Largely preventable chronic diseases such as cardiovascular disease, stroke, diabetes and obesity and are now the major causes of death and disability worldwide. They are also now rapidly spreading throughout the developing world.

In 2003 the annual report of the World Health Organisation listed physical inactivity among the main risks contributing to global chronic disease morbidity and mortality. Physical inactivity was estimated to cause 1.9 million deaths in 2000 and 19 million disability adjusted life year losses annually. Inactivity was also implicated in about 15%-20% of cases of ischaemic heart disease, diabetes and some cancers. Worldwide, it is estimated that over 60% of adults are simply not active enough to benefit their health.

The benefits of physical activity and exercise have never been so clear. This edition of the *Trinity Student Medical Journal* comprehensively demonstrates the benefits of exercise in relation to diabetes, cholesterol and osteoporosis. It is clear that physical activity is a strong means by which individuals can prevent serious disease, and a cost-effective way by which societies can improve public health.

The need for us to exercise has never been greater. The encouragement from governments, public health organisations and voluntary organisations has never been so strong, yet despite both these factors, participation has never been so low.

It is one thing to know and understand the benefits of exercise. The question now becomes how we utilise that knowledge. How do we alter behaviour? Telling the public that exercise is good for them is not enough. Evidence demonstrates that knowledge of the benefits of exercise does not predict exercise behaviour. The evidence also suggests that scare tactics alone are generally ineffective. Individuals rarely change their behaviour in response to coercion or unduly paternalistic messages. Coercion, in fact, may lead to resistance. Marketing campaigns designed to influence lifestyle have been most successful in changing behaviour among people with higher levels of education and income. While these campaigns might be marginally successful, they have been least effective for the most disadvantaged populations. They may in fact have the unintended effect of increasing health inequality between socio-economic groups.

A review of available evidence does not provide a clear indication as to which health and exercise promotion strategies are most effective. We seem to have more information about what health behaviours need to be changed and what is not effective, than a clear picture as to how to proceed from here.

The battle to ban smoking in the workplace was hard fought. It may however pale by comparison with the task ahead. We know what health behaviours need to be changed. The real challenge will be to bring about that change.

Oesophageal Carcinoma: A Review

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4th Year Physiology

INTRODUCTION

Oesophageal cancer is one of the most lethal forms of gastrointestinal (GI) cancer with only a 9% five-year survival rate currently in the United States (US).[1] It is the ninth most common cancer worldwide and has shown an increasing incidence in Western civilisation in recent decades, coinciding with a striking shift in histologic type and primary tumour location.[2] It is curable in its earliest stages, however it usually presents as an advanced disease.[1] In terms of cancer sites characterised by poor survival, it ranks fourth, behind carcinomas of the liver, pancreas and lung.[1] Despite the last two decades of clinical research, the median survival time for the patient with symptoms of a primary oesophageal cancer is less than 18 months.[3]

EPIDEMIOLOGY

Cancer of the oesophagus has the greatest variation in geographic distribution of any malignancy with highest rates being reported in South Africa, China, Brazil and Japan.[4] In the US, it is the fourth leading cause of cancer death among African American males and the seventh among Caucasians, being three times more common in men than in women.[3] In 2003 the American Cancer Society estimated that approximately 13,900 new oesophageal cancer cases would be diagnosed in the US (10,600 men, 3,300 women) and that 13,000 deaths from the disease would occur (9,900 men, 3,100 women).[5]

In Ireland between 1994 and 1996, an average of 445 new cases were diagnosed per year (268 male, 177 female) with an average of 441 deaths per year (266 male, 175 female), with the age standardised incidence and mortality rates for oesophageal cancer being about twice as high in males than in females. Rates of oesophageal cancer in females in Ireland were reported to be almost three times the EU average, and in males were substantially higher than the EU average.[6]

In America, the overall incidence of and mortality from oesophageal cancer has increased by about 15% over the past 3 decades (Figure 1), with the increase mainly being due to adenocarcinomas of the lower oesophagus and gastroesophageal junction.[1,7] Currently, the incidence of oesophageal adenocarcinoma has a rate of increase greater than that of any other cancer in the United States.[3] Although oesophageal squamous cell carcinoma remains the predominant type of oesophageal malignancy in the remainder of the world, adenocarcinoma is now the tumour with the fastest increasing incidence at a rate of 10% per year, the increase being most dramatic among Caucasian males.[8]

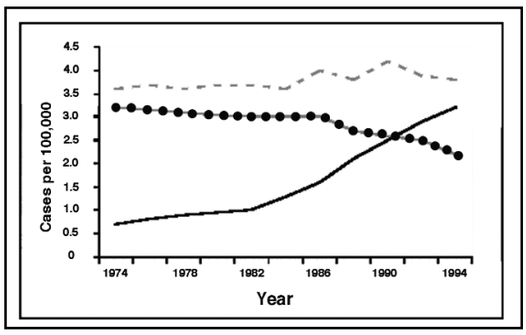

Figure 1. The increase in the incidence of adenocarcinoma in the US over the past several decades.Dashed line: all oesophageal cancers, in all races, men and women; dotted line: squamous cell carcinoma in Caucasian men; solid line: adenocarcinoma in Caucasian men. Data from Spechler[7] and Ries *et al*.[1]

Improvements in diagnostic techniques and changes in cancer classification may explain some of the rise in reported incidence rates, but detection bias and misclassification bias do not appear adequate to explain the increase entirely.

In Asia oesophageal cancers are predominantly of the squamous cell type, and mostly located in the middle third of the oesophagus. In these populations there has been no notable rise in the incidence of adenocarcinoma of the oesophagus and gastric cardia.[9]

PATHOLOGY

More than 90% of oesophageal cancers are either squamous cell carcinomas or adenocarcinomas. On rare occasions other carcinomas, melanomas, leiomyosarcomas and lymphomas may develop as well. Squamous cell (epidermoid) carcinoma arises from the mucosa of the oesophagus. Histologically, it is characterised by invasive sheets of polygonal, oval or spindle shaped cells that run together and are cohesive, with a distinct ragged stromal epithelial surface.

Adenocarcinoma by definition is a carcinoma derived from glandular tissue or in which the tumour cells form recognizable glandular structures. This gland-like or gland-derived carcinoma arises from three sources: superficial and deep glands of the oesophagus such as mucous glands, embryonic remnants of glandular epithelium and metaplastic glandular epithelium. Approximately 75% of all adenocarcinomas are found in the distal oesophagus whereas squamous cell carcinomas

are more evenly distributed between the middle and lower third.[10]

AETIOLOGY

In general, a high dietary intake of fruits and vegetables have been shown to have a protective effect against oesophageal cancer. High levels of vitamins A and C and riboflavin have been suggested as the responsible protective factors.[11] Certain epidemiological studies have suggested that regular aspirin therapy may also protect against the disease.[12]

A high fat diet along with tobacco smoking and high alcohol intake will increase the risk of disease. Studies have shown the risk of oesophageal cancer correlates directly with the quantity of cigarettes smoked per day and the duration of smoking.[13] A history of mediastinal radiotherapy, such as for the treatment of breast cancer, also predisposes the patient to both histologic types of oesophageal cancer.[14]

Any factor that causes chronic irritation and inflammation of the oesophageal mucosa appears to increase the incidence of squamous cell carcinoma.

Squamous cell carcinoma, but not adenocarcinoma, is clearly linked to low socioeconomic status.[14] Several conditions such as oesophageal diverticula, oesophageal webs and tylosis (a rare autosomal dominant disorder) predispose patients to squamous cell carcinoma. In fact, in affected families tylosis confers up to a 95% risk of squamous cell carcinoma by 70 years of age.[14]

The major risk factor for oesophageal adenocarcinoma is gastroesophageal reflux disease (GORD) and its sequela, Barrett's oesophagus. In Barrett's oesophagus, squamous epithelium damaged by reflux oesophagitis is replaced by a metaplastic epithelium containing several cell types with gastric, small intestinal or colonic features. This metaplastic epithelium can be viewed teleologically as the body's way of protecting tissues from a hostile environment. The problem is that these cells are predisposed to develop DNA alterations that lead to dysplasia and cancer.[15] Barrett's oesophagus occurs in 5%-8% of patients with GORD and for reasons that are not entirely clear, its prevalence has increased from an average of 1 per 1000 upper endoscopies in the early 1980s, to over 55 to 60 per 1000 in the late 1990s. Prospective studies on the incidence of adenocarcinoma in Barrett's oesophagus have provided risk estimates ranging from 0.4% to almost 2% per year.[7]

A recent epidemiological study conducted in Sweden found that patients who experienced heartburn, regurgitation, or both, at least once a week had a risk of developing adenocarcinoma that was increased nearly eight-fold above that of asymptomatic subjects in the general population.[15]

Factors that May Contribute to Barrett's Oesophagus and Adenocarcinomas

The parallel epidemic of obesity, which may increase intraabdominal pressure and thus predispose to GORD, has been implicated. In fact several epidemiologic studies have shown upwards of three-fold excess risk among overweight individuals.[16] The prevalence of *Helicobacter pylori (H. pylori)* infection is steadily decreasing in Europe and the US, and this parallels an increase in GORD and adenocarcinomas of the oesophagus and oesophagogastric junction. It has been postulated that *H. pylori* infection (particularly strains that are positive for the *cagA* protein) may reduce the risk of GORD by reducing gastric acidity.[17]

Supporting this theory, it has been shown that in regions with a higher prevalence of *H. pylori* infection such as in Asia, the percentage of adenocarcinoma is much smaller.[18]

Factors such as hiatial hernia, increased use of lower oesophageal sphincter-relaxing medications and a family history of breast cancer have also been implicated.[14]

MOLECULAR AND CELLULAR CHANGES

During the development of oesophageal cancer there is progression from a premalignant epithelium to a neoplasm that frequently demonstrates a heterogeneous mix of genetic alterations. In the vast majority of oesophageal cancers inactivation of the p53 and p16 genes at an early stage is followed by defects in genes such as retinoblastoma (Rb) and cyclin D1 and E at later stages.

Amplification of the *c-erb* gene is a prognostic factor and predictive of lymph node involvement.[19] Loss of heterogenicity (LOH) of 17p, the p53 locus, has been detected in 52% to 93% of adenocarcinomas in Barrett's oesophagus and in squamous cell carcinomas. P53 alterations have been detected in 55% to 76% of cases.[20] Additionally, several European and North American studies have shown an association between p53 mutations and smoking.

The *Erb* family of receptor tyrosine kinases and their growth factor ligands, epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α), have also been implicated with increased expression of TGF-α and the EGF receptor being detected in Barrett's metaplasia.[21] This is seen in both oesophageal adenocarcinoma and squamous cell carcinomas.[21]

The overexpression of cycloxygenase (COX) enzyme has also been shown to contribute to the process of apoptotic resistance of oesophageal cancer cells. Overexpression of COX-2 has been detected in oesophageal carcinomas and recent studies have shown that selective COX-2 inhibitors significantly decrease proliferation and increase apoptosis *in vitro* in oesophageal squamous and adenocarcinoma cell lines.[20]

CLINICAL FEATURES

Like other cancers of the GI system, oesophageal cancers are rarely found early when they are small and more easily treated. More frequently, the patient presents late because the distensible oesophagus compensates readily for partial obstruction of the lumen by a tumour. The tumours are characterised by extensive local growth, lymph node metastasis and invasion of adjacent structures before wider dissemination. The poor prognosis of oesophageal cancer patients is influenced by the proximity of the aorta and trachea and the absence of a serosal covering.

The typical presenting patient is a male between 55-65 years old with a long-standing history of cigarette abuse and heavy alcohol intake. Dysphagia and weight loss are the initial symptoms in 75% of cases.[10]

Most patients complain of food sticking at a point in their throat at the level of the sternal notch. Odynophagia is seen in about 25% of the patients with tumours.[10] Regurgitation of undigested food, retrosternal or epigastric pain or aspiration pneumonia may be present. Advanced lesions may present with haematemesis, melaena, cough from a tracheoesophageal fistula, haemoptysis or problems related to nerve involvement. Tumours of the oesophagus may present with superior vena cava syndrome but this is rare in the absence of dysphagia.

DIAGNOSIS

Endoscopy is usually the first diagnostic test in people with suspected oesophageal cancer. Early cancers can be detected this way but are usually incidental findings. Following this, a computed tomographic (CT) scan of the chest, abdomen and pelvis with intravenous contrast medium is preformed to detect metastasic disease.[14]

More recent advances in diagnosis include endoscopic ultrasonography (EUS) and positron emission tomography (PET). EUS is a procedure that, according to recent studies, might be even more accurate than CT scans and endoscopy in determining the size of an oesophageal cancer and the degree of invasion.[22]

Positron emission tomography (PET) is being described as the most promising non-invasive procedure for the evaluation and management of patients with oesophageal neoplasms and is beginning to replace invasive thoracoscopic and laparoscopic staging in many institutions. PET has been shown to be accurate in the identification of primary oesophageal neoplasms of either histologic type, to be more accurate than CT in preoperative evaluation for the identification of distant metastatic disease and also to be more accurate as a restaging procedure for the detection of regional and distant site recurrance.[23,24]

Fusion of CT or magnetic resonance images (MRI) with PET images also shows great promise as a valuable tool in helping to deliver more accurate staging and yield better sparing of normal tissue.

SCREENING AND SURVEILLANCE

The poor prognosis and lethal nature of oesophageal cancer along with the lack of efficacy of chemotherapy and radiation therapy provides the rationale for promoting surveillance endoscopy in patients with chronic GORD symptoms, particularly those with Barrett's oesophagus. It is known that progression from metaplasia to invasive cancer occurs in a stepwise process,[25] so it is reasonable to assume that effective surveillance programs such as that outlined in figure 2 can be developed for patients known to have Barrett's oesophagus.

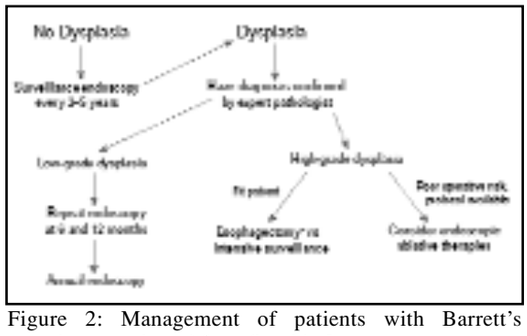

Figure 2: Management of patients with Barrett's oesophagus. Adapted from Spechler[7]

Indirect evidence suggests that surveillance for Barrett's oesophagus may be beneficial. Several studies have shown that patients whose oesophageal carcinomas are discovered during surveillance for Barrett's oesophagus have tumours in earlier stages and have better survival rates than patients whose cancers are discovered because they have symptoms such as dysphagia and weight loss.[7]

All opposing argument is that the relatively low incidence, absence of early symptoms and the

rarity of a hereditary form of the disease make population based screening untenable except in certain high-risk areas of the world.

TREATMENTS

Early Forms of the Disease: Barrett's Oesophagus and High-Grade Dysplasia

The management of patients with Barrett's oesophagus with high-grade dysplasia is controversial. Those who are good operative candidates have traditionally been offered surgical resection because of the high risk of adenocarcinoma. However, surgery is associated with significant mortality.[14]

An alternative method is based upon the observation that the destruction of intestinal metaplasia using a variety of thermal and chemical methods may be accompanied by regrowth of normal appearing squamous epithelium, particularly if the patients are treated with proton pump inhibitors.

Endoscopic mucosal ablative therapies would seem to be ideally suited for the ablation of dysplastic Barrett's epithelium and early oesophageal cancer because these diseases are localised to the oesophageal mucosa. Therapies that have been specifically used for Barrett's with high grade dysplasia include: photodynamic therapy (PDT), laser ablation (Nd:YAG), cryotherapy and endoscopic mucosal resection (EMS). The combination of EMS and PDT has been shown to be an effective and safe therapy and appears to be the most promising approach, but the long-term effects of ablative therapy are not known and continued surveillance is still advised.[26] Data on the use of PDT for Barrett's oesophagus seems to be slightly more favourable than those for thermoablation.[27]

Localised Cancer

Survival figures for surgery have improved in recent decades due to earlier diagnosis, more accurate staging, prudent patient selection and altered surgical techniques. A recent multi-institutional randomised trial reported that patients undergoing surgery as the sole treatment had median survival rates ranging from 13 to19 months, 2 year survival rates ranging from 35% to 42%, and 5 year survival rates from 15% to 24%.[14]

To improve outcomes clinical trials have assessed the role of other modalities of treatment in conjunction with surgery. The combination of cisplatin and 5-flurouracil (5-FU) are considered the optimal chemotherapy agents, but results of trials are conflicting as to the benefit of preoperative therapy. Radiation therapy alone either pre- or postoperatively has also failed to improve survival indices. Postoperative radiotherapy has even been shown to have a detrimental effect on survival.[28]

At least eight randomised trials have been conducted to address the potential benefit of preoperative chemotherapy with radiotherapy. Only two studies enrolled sufficient numbers of patients to provide statistically meaningful results and neither reported an advantage of neoadjuvant combined modality therapy.[14] The one positive trial by a group from St James's Hospital and Trinity College in 1996 showed a benefit of combined therapy.[29] This trial came under criticism for small numbers and poor surgical outcome but appears to have influenced thoracic surgeons and oncologists substantially.

Postoperative combined treatment is frequently offered to patients whose tumour cells extend to the surgical margin but there is no documented evidence that postoperative chemotherapy or radiotherapy is beneficial in the absence of residual disease.[14]

Chemotherapy and radiotherapy have been shown to lead to long-term survival in 25% of patients, an outcome similar to that associated with surgery alone or surgery after preoperative therapy.[29]

Advanced Metastatic Disease

In patients with distant metastases a more palliative approach should be considered. Local symptoms such as relief from dysphagia may be controlled with oesophageal stents, external beam radiotherapy, brachytherapy or mucosal ablative therapies. The results of small randomised trials comparing stenting, laser ablation and PDT suggest that stenting offers a similar degree of relief from dysphagia and at a lower cost but may cause severe acid reflux and tumour ingrowth.[30]

Although chemotherapy can palliate symptoms in many patients, the response to it typically lasts no longer than a few months and survival is short, rarely exceeding one year. Combination chemotherapy is likely to improve response rates over single agent therapy but this may not translate into a significant survival benefit.[31]

FUTURE OUTLOOK

As the epidemiology of oesophageal cancer evolves, current practice must change accordingly to keep pace with evidence based data so as to treat patients with the best option available. Progress with newer chemotherapeutic agents, optimal radiotherapy prescriptions and/or innovations and alternative forms of systemic treatment merit further investigation. The elucidation of the basic mechanisms of oesophageal carcinogenesis brings with it the

promise of developing treatment and preventive strategies that are based on the molecular biology of these tumours, such as antagonists of the EGF receptor or COX-2-inhibitors.

In Ireland, further investigation is needed into the possible factors accounting for the disproportionately high rates of oesophageal cancer among women. Screening initiatives for high-risk groups should also be considered.

The population should be encouraged to stop smoking, eat a diet with a high level of fresh fruit and vegetables and moderate alcohol consumption.

One of the major issues surrounding the disease is that patients often do not understand the potential significance of their symptoms and present quite late, often too late. It is the job of health care professionals and the government to educate and inform people more about the disease so as to increase awareness of the primary symptoms such as dysphagia and weight loss and reduce the incidence of this deadly disease.

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Cardiovascular Complications of HIV Infection

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5th year Medicine

INTRODUCTION

Rapid development in the management and treatment of HIV infection has lengthened the lifespan of this patient population, but the increased frequency of cardiovascular diseases related to increased lifespan has emerged as an added potential burden to the cost of their healthcare.[1] Cardiovascular diseases commonly associated with HIV infection include pericardial effusion, dilated cardiomyopathy, endocarditis, coronary artery disease, systemic arterial hypertension, pulmonary hypertension, malignant neoplasms, and drug-related cardiotoxicity. This review discusses recent insights into the pathophysiology of cardiovascular diseases in HIV-infected patients.

In recent years, effective therapies for HIV-infected patients have led to increased survival and a longer lifespan. This has led to the increased recognition of the manifestations of late-stage HIV infection, including HIV-related cardiovascular diseases. The prevalence of cardiovascular disease in HIV/AIDS has a reported range of between 28% and 73%,[1] which includes manifestations such as pericardial effusion, dilated cardiomyopathy, myocarditis, endocarditis, coronary artery disease, systemic arterial hypertension, pulmonary hypertension, malignant neoplasms, and drug-related cardiotoxicity. The pathogenesis is multifactorial, and may result from the viral infection itself, opportunistic infections complicating the immunodeficiency, nutritional deficiencies, autoimmunity or drug therapy.[2] Dyslipidaemia associated with anti-retroviral therapy has recently emerged as an issue of concern as it possibly increases the risk of premature atherosclerosis and cardiovascular disease.[2,3]

PERICARDIAL EFFUSION

Pericardial effusion is common in patients with HIV (estimated to be up to 11% per year)[5] and has a variable presentation. It may be asymptomatic or manifest as pericarditis or as cardiac tamponade.[4]

This incidence was studied by Heidenreich *et al* who showed an increasing trend of pericardial effusion with progression of HIV infection (asymptomatic HIV positive patients showed an incidence of 0% whereas patients with AIDS had an incidence of 11% per year). This study also demonstrated a ninefold increase in 6-month mortality in HIV patients diagnosed with pericardial effusions compared to patients without effusions. Although a definitive cause is often not found, it may be related to opportunistic infections, malignancy or a cytokine mediated capillary leak syndrome seen in end-stage HIV disease (see Table 1).[5]

A low CD4 count is observed in HIV patients with pericardial effusions which reflects the functional status of their immune system. The effusion may serve as a marker for end-stage HIV infection associated with undiagnosed opportunistic infections or malignant neoplasms. In up to 42% of patients a pericardial effusion will spontaneously resolve and therefore, pericardiocentesis is only performed diagnostically or to relieve poorly tolerated effusions. However, despite this spontaneous resolution, the patient's mortality remains increased once an effusion has developed.[5]

DILATED CARDIOMYOPATHY

Dilated cardiomyopathy, a primary cardiac muscle disease associated with severe left ventricular dysfunction, is the most common life-threatening cardiovascular complication of HIV infection.[6] It has an estimated annual incidence of 15.9 per 1000 before highly active antiretroviral therapy (HAART), and its prevalence (determined by echocardiographic and autopsy studies) ranges from 10-30%,[7] with echocardiographic findings of four-chamber enlargement, left ventricular hypokinesis and decreased fractional shortening.[8] At post mortem gross findings include increased heart weight and a pale myocardium.[9]

Although some studies have shown a role for direct HIV-1-mediated injury of the myocardium,[10] the exact pathogenesis of cardiomyopathy is still unknown. HIV-1 infection of the myocardium seems to occur in a patchy distribution without any definite association between HIV-1 and cardiac myocyte dysfunction.[11] Coinfection with Coxsackie group B virus, *Cytomegalovirus* and Epstein-Barr virus has been observed in some HIV patients and these may be implicated in the pathogenesis of cardiomyopathy (Table 1).[12]

Autoimmune reactions may play a key role in the pathogenesis of HIV-associated cardiomyopathy as circulating cardiac autoantibodies (anti-α myosin autoantibodies) have been reported in up to 30% of patients.[13]

Therefore in HIV-positive patients with previously normal echocardiographic findings, these autoantibodies could then serve as a marker for left ventricular dysfunction.[13] Another marker which can potentially be used for detection is brain natriuretic peptide (BNP) as there has been growing evidence to support the hypothesis of increased levels of this peptide in association with HIV-related cardiomyopathy.[14]

Malnutrition has also been implicated in contributing to ventricular dysfunction in cardiomyopathy. An improvement of cardiac function was seen after selenium supplementation in case reports of pediatric AIDS patients and there may also be alteration in levels of vitamin B_{12}, carnitine, growth hormone and thyroid hormone in patients with HIV disease (Table 1) which have all been associated with left ventricular dysfunction independantly.[15,16]

Evidence to suggest a beneficial effect of HAART on HIV-associated cardiomyopathy has yet to emerge. In contrast, Zidovudine has shown to be associated with mitochondrial DNA replication inhibition and cardiac mitochondrial ultrastructure destruction by studies on transgenic mice. This mitochondrial dysfunction is linked to a lactic acidosis, which may further deteriorate myocardial function.[17]

Intravenous immunoglobulin has been shown to be efficacious as a specific therapeutic regimen for HIV-associated cardiomyopathy with a 10% improvement in contractility and a 15% improvement in peak wall stress in children who receive this treatment. This supports the theory that the ventricular dysfunction seen in HIV-associated cardiomyopathy is mediated by immunological means and would therefore be responsive to immunomodulatory therapy.[18]

ENDOCARDITIS

Adherent and friable vegetations of platelets and red blood cells characterize marantic endocarditis, or nonbacterial thrombotic endocarditis, which is seen in 3% to 5% of AIDS patients.[19] It is usually found in patients older than 50 years, and in association with the HIV-wasting syndrome.[20] Systemic embolization involving the brain, lung, spleen, kidney and coronary arteries may occur in up to 42% of patients.[21] This is, however, a rare cause of death in AIDS patients.[11]

Infective endocarditis in HIV-infected patients is most commonly seen to affect the tricuspid valve (this is probably because of the association of HIV with intravenous drug misuse) and has an estimated prevalence of 6.3% to 34% in this population. Causative organisms include *Staphylococcus aureus* (more than 75% of cases), *Streptococcus viridans*, *Hemophilus influenzae*, *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*.[20] In general, the presentation and survival of patients with infective endocarditis is similar between HIV positive and HIV negative patients (85% versus 93%).[20] However, in the late stage of HIV-infected patients, a significantly increased mortality (30%) has been reported.[22]

CORONARY ARTERY DISEASE

An increasing frequency of coronary artery disease has been reported in patients with HIV who are taking protease inhibitors (PIs) as a part of the HAART regimen.[11] Class-specific metabolic side effects of PIs such as dyslipidaemia and insulin resistance add to pre-existing cardiovascular risk factors and contribute to premature arteriosclerosis. In addition to this, HIV associated chronic inflammation with increased levels of C-reactive protein may accelerate atherosclerosis in these patients.[23] A study by Dressman *et al*[24] on the effects of PIs in mice showed that PIs induce CD36 gene expression on macrophages, which promotes the development of foam cells and subsequent atherosclerosis. The induction of CD36 on macrophages is related to the activation of peroxisome proliferator-activated receptor-γ (PPARγ), opening up therapeutic options for the use of thiazolidinediones (PPARγ inhibitors) to modulate the proliferative & inflammatory cascades involved in the atherogenic process.[25]

It has been suggested in recent findings that non-nucleoside reverse transcriptase inhibitors (NNRTIs) may increase the levels of high-density lipoprotein (HDL) cholesterol and these drugs would therefore be expected to be associated with decreased coronary artery disease incidence.[26] A HAART regimen based on NNRTIs may then be preferred in patients who are at high risk of coronary artery disease.

No correlation has been found between the development and progression of coronary artery disease and a patient's CD4 count or HIV-related opportunistic infections. However, on histology endoluminal protrusions resulting from diffuse vessel involvement of smooth muscle cell proliferation with an abundance of elastic fibers can be seen and this is a unique characteristic of HIV-related coronary arteriopathy.[27]

The incidence of acute myocardial infarction in HIV-infected patients has been reported at 5-5.5 per 1000 person-years, a threefold increase compared to non HIV-infected patients (1.52 per 1000 person-years).[28] Patients are predominantly male, with an average age of less than 50 years, and have a benign in-hospital course. However, after discharge HIV-infected

patients have a higher incidence of re-infarction, restenosis and stent thrombosis.[29]

SYSTEMIC HYPERTENSION AND COAGULATIVE DISORDERS

Elevated blood pressure has been linked to metabolic disorders and the lipodystrophy induced by protease inhibitors (Table 1).[30] With an estimated prevalence of 20% to 25% before the introduction of HAART,[31] systemic hypertension now has a prevalence of up to 74% in patients with HAART-related metabolic syndrome.[30] An increased risk of coagulative disorders is an issue of concern in patients receiving protease inhibitor-containing HAART therapy that include a protein S deficiency, increased levels of fibrinogen, D-dimer, plasminogen activator inhibitor-1 and tissue-type plasminogen activator antigen.[32,33] Documented thromboses in both veins and arteries have been associated with these disorders.[33]

PULMONARY HYPERTENSION

The incidence of pulmonary hypertension associated with HIV had been reported at 1 in 200 compared with 1 in 200,000 in the general population before the introduction of HAART.[34] It is seen most frequently in young male patients and is associated with intravenous drug users, homosexuals and haemophiliacs. Symptoms can range from dyspnoea to right-sided heart failure, cor pulmonale or death.[35]

The pathogenesis of the pulmonary arterial disease associated with HIV infection is unknown, and the hypothesis of a direct impact of the virus on the pulmonary vascular smooth muscle and/or endothelial cells has not been demonstrated.[36] However, histology frequently shows the presence of HIV-1 in alveolar macrophages which in turn release proteolytic enzymes, oxide anions and TNF-α in response to infection.[35] This supports the theory of HIV's indirect role in producing growth factors which lead to abnormal endothelial and smooth muscle cell proliferation.[37] The current treatment regime includes anti-coagulation and the use of vasodilator agents such as epoprostenol which is limited to use in very ill patients.[38] The effects of HAART on pulmonary hypertension in HIV-infected patients remains unknown.

MALIGNANT NEOPLASMS

Kaposi's sarcoma involving the heart in AIDS patients has an estimated incidence of 12% to 28% in retrospective autopsy studies.[20] Such cardiac involvement usually occurs as a part of disseminated Kaposi's sarcoma and is not usually linked to cardiac dysfunction, morbidity, or mortality.[11]

Malignant lymphoma in AIDS is usually derived from B cells, is typically of a high grade nature and disseminates early.[20] A primary cardiac lymphoma may also occur although this is extremely rare.[39] Patients present with a varied spectrum of clinical manifestations ranging from non-specific symptoms to congestive heart failure, pericardial effusion, cardiac arrhythmia or cardiac tamponade.[40,41,42] Despite the poor prognosis of patients with HIV-associated cardiac lymphoma, combination chemotherapy has yielded clinical remission in some cases.[43]

DRUG-RELATED CARDIOTOXICITY

The many medications to which HIV-infected patients are exposed in treating HIV-associated diseases (such as cancer and opportunistic infections) allows much more opportunity for drug toxicities and interactions (Table 2). Dilated cardiomyopathy has been seen in patients on amphotericin B, doxorubicin and foscarnet sodium.[44,45,46] Hypertension has been associated with erythropoeitin therapy (47% prevalence) and cardiotoxicity has been reported with interferon alpha in a review of cases by Sonnenblick and Rosin.[47,48] The most common manifestation of cardiotoxicity was arrhythmia, followed by myocardial infarct, cardiomyopathy, sudden death, AV block and congestive heart failure. Ganciclovir has been associated with ventricular tachycardia and QT prolongation has been seen with the use of pentamidine, pyrimethamine and trimethoprim-sulfamethoxazole.[49,50,51,52]

RISK STRATIFICATION FOR HIV-INFECTED PATIENTS

With the frequency of cardiovascular complications seen in the HIV-infected population, appropriate preventive screening and therapeutic strategies should be implemented in order to improve the quality of life and survival in this group of individuals. Dilated cardiomyopathy can be visualized and diagnosed by echocardiography and has been shown in association with increased levels of BNP.[14] In a study by Hervas *et al*, BNP showed a positive correlation with ventricular diameter and pulmonary artery systolic pressure and may, therefore, also play a role in the identification of patients with pulmonary hypertension.[53]

As NNRTIs have shown to be potentially beneficial on lipid profiles, a NNRTI-based HAART regimen may be favourable in patients who have an increased cardiovascular risk.[26] PI-containing HAART has been associated with vascular risk factors such as dyslipidaemia and metabolic syndrome, and therefore all patients

receiving HAART should be assessed for lipid and other metabolic abnormalities both before and at regular intervals after initiation of therapy.

Lipid abnormalities can be treated by dietary and pharmacological means with potential drug interactions taken into consideration.[54] Fibrates and statins are useful in lowering cholesterol and triglyceride levels although it should be noted that many statins are metabolized by the cytochrome P450 CYP3A4 enzyme pathway which is inhibited by protease inhibitors. This interaction may lead to a several fold increase in statin concentration and potentiate the risk of hepatic and skeletal muscle toxicity.[11]

CONCLUSION

Aggressive use of HAART therapy regimens for HIV-infected patients has decreased the incidence of opportunistic infection, pericardial effusions and HIV-associated malignancies which has helped to maximize patient survival and quality of life. Evidence has shown that protease inhibitors play a role in promoting and accelerating atherosclerosis and coronary heart disease which increases the mortality from myocardial infarction and effects of cerebrovascular disease such as stroke. To prevent the significant morbidity and mortality from cardiac involvement associated with HIV, prompt intervention of cardiac disease with screening and early recognition is important.

Table 1. Major cardiovascular manifestations associated with HIV-infection.

Type	Incidence	Cause
Pericardial Effusion	11% per year (5)	Infective: Bacterial, Mycobacterial, Viral, Fungal Malignancy: Kaposi's sarcoma, Malignant lymphoma Hypothyroidism Malnutrition Cytokine mediated capillary leak
Dilated Cardiomyopathy	1.59% before the introduction of HAART	Infective: HIV, Coxsackievirus Group B, Epstein-Barr Virus, Cytomegalovirus Autoimmune Nutritional deficiency Selenium, Vitamin B12, Carnitine Endocrine Thyroid hormone, growth hormone Hypothermia/hyperthermia High HIV viral load, immunosuppression
Coronary Artery Disease	Case reports limited to HAART with use of Pis	Adverse effects of protease inhibitors: Metabolic, Coagulative Arteritis
Systemic hypertension	20%-25% before introduction of HAART. Up to 74% in relation to HAART metabolic syndrome	HIV-induced endothelial dysfunction Premature atherosclerosis secondary to HAART Adverse effects of protease inhibitors: Insulin resistance, increased sympathetic Activity, sodium retention
Pulmonary hypertension	0.5% before the introduction of HAART	Mediators released from the endothelium Recurrent bronchial infections Multiple pulmonary emboli fragments (possibly from endocarditis)
Malignant tumors	12%-28% before introduction of HAART	Kaposi's Sarcoma Non-Hodgkin lymphomas

Table 2. Cardiovascular toxicities associated with common HIV medications.

Drug	Use	Cardiac Side Effects
Amphotericin B	Anti fungal	Dilated Cardiomyopathy, hypertension, bradycardia
Doxorubicin	Kaposi's sarcoma Non-Hodgkin's lymphoma	Dose related dilated cardiomyopathy
Erythropoeitin	Anemia	Hypertension
Foscarnet sodium	Cytomegalovirus (CMV) Esophagitis	Dilated cardiomyopathy
Ganciclovir	CMV	Ventricular tachycardia
Interferon alfa	Anti neoplastic, anti viral	Arrhythmia, myocardial infarction, cardiomyopathy, sudden death, AV block, congestive heart failure
Pentamidine	*Pneumocystis carinii*	QT prolongation, Torsades de pointes, severe hypotension
Pyrimethamine	Toxoplasmosis	QT prolongation
Trimethoprim-sulfamethoxazole	*P. carinii*	QT prolongation, Torsades de pointes
Zidovudine	Anti retroviral	Myocarditis, dilated cardiomyopathy

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A New Method to Quantify Mitral Regurgitation

Sandeep Bhachu

INTRODUCTION

In cases of mitral regurgitation, the severity of the regurgitation is the major determinant of progression to left ventricular dilatation and dysfunction. Therefore, precise measurement of regurgitant volume in patients with mitral regurgitation is important for evaluating the progression of the disease which can then determine the optimal time for surgical repair or replacement.[1] A wide range of analytic approaches and diagnostic technologies have been proposed to aid clinical evaluation of mitral regurgitation. Yet all existing quantification methods have shown limitations in one form or another. The aim of this paper is to present a new technique based on a control volume method which can accurately quantify mitral regurgitation with the use of velocity encoded magnetic resonance images.

MITRAL REGURGITATION

Mitral valve regurgitation, also known as mitral insufficiency, is a disorder in which the mitral heart valve does not close properly, causing blood to regurgitate into the left atrium when the left ventricle contracts. As a result, the left ventricle has to pump more blood to make up for the leakage and thus gradually enlarges to increase the force of each heartbeat. The left atrium also tends to enlarge to accommodate the extra blood being pushed back from the ventricle.[2] However, profound enlargement of the atrium often results in atrial fibrillation, triggering the ventricles to contract more rapidly and reducing the heart's pumping efficiency. The lack of proper blood flow through the ventricle allows blood clots to form which, if dislodged, will be pumped out of the heart and may block a smaller artery possibly causing a stroke or other damage. Finally, severe regurgitation reduces the forward flow of blood sufficiently to cause heart failure and death.[3]

Although medication may temporarily decrease symptoms, the only proven treatment for mitral regurgitation is surgical repair or replacement of the valve. There have been significant advances in the way mitral valve surgery is performed, and in otherwise relatively healthy patients, the risks for major complications including death are relatively low, in the order of 3-5%.[4] Even though surgical correction is highly successful, the operative risk in valve repair or replacement, despite recent improvements, is still far from negligible. Therefore surgery, particularly for patients with minimal or no symptoms, should be considered only for patients with well-documented severe mitral regurgitation.[1] Consequently, determining the degree of regurgitation is a crucial part of the clinical evaluation of patients with mitral regurgitation.

Throughout the past decade, there has been a great deal of research into the quantification of mitral regurgitation to determine the level of severity of the disease.[5] A wide range of analytic approaches and quantification techniques have been proposed to aid clinical evaluation and decision-making. However, mitral regurgitation currently has no gold standard against which to determine the regurgitant volume.[6] Thus, the optimal management of patients with the disease is still controversial. The two most commonly used quantification techniques to date are left ventriculography and cardiac catherisation, and colour doppler echocardiography. Each approach has limitations and inaccuracies. For instance, left ventriculography and cardiac catherisation is invasive, time-consuming and is not feasible for repeated measurements since it has inherent risks. In addition, in order to calculate the heart's ejection fraction it utilizes assumptions concerning the properties of the left ventricle that may not be true.[7,8]

Colour doppler echocardiography can determine and display the direction and velocity of blood flow in the heart chambers and vessels and subsequently produce an image of the regurgitant jet. Although this technique allows one to establish the presence of mitral regurgitation, the quantification is imprecise.[8] Essentially, the technique attempts to solve a three-dimensional problem from a two-dimensional image by measuring the jet area.[9] A correlation does exist between size and volume flow. However, it is not perfect and thus masks the true volume of blood flow back through the mitral valve.[10]

Thus, the quantification of mitral regurgitation continues to remain an important but elusive clinical goal. With today's technological advances, the goal of improving and developing new techniques is within our grasp. Ideally, an accurate, rapid, non-invasive and widely available technique for quantifying mitral regurgitation is desirable. A more accurate classification of the disease severity would enable the medical

community to predict disease progression more accurately and plan more timely surgical interventions. Such a quantification technique may exist with the use of magnetic resonance imaging (MRI).[11,12]

CONTROL VOLUME THEORY

In order to study a phenomenon such as the regurgitant volume, the physical system must be isolated from its surroundings and mathematical models that describe it must be developed. It is here that we utilise the concept of a control volume.[13]

A control volume can be thought of as a finite region, chosen carefully by the analyst, with open boundaries through which mass, momentum, energy are allowed to cross. The analyst will then be able to determine the balance between the incoming and outgoing fluid, and the resultant changes within the control volume. The result is a powerful tool.

In a recent study, the quantification of aortic regurgitation has been shown to be possible if a single MRI phase velocity encoded slice is positioned perpendicular in the aorta close to the aortic valve.[12] This type of MRI image is unconventional in that its intensity is independent of signal magnitude but directly proportional to blood flow velocity.[14] Thus, one can measure the velocity of blood flowing forward and backward through the aortic valve. This process, which in the past has been referred to as the single slice method is appropriate for the aortic valve as the aortic flow field and anatomy are relatively simple during the period of regurgitation. Simply speaking, aortic regurgitation can be isolated by a single plane placed through the aortic sinus allowing the regurgitation flow to be calculated accurately. Once the flow rate (velocity x area) has been determined, integration of the aortic flow curve over the diastolic period can then provide the regurgitant volume per cardiac cycle.[15] However, this method cannot be used in the same manner for mitral regurgitation. Unfortunately, for the quantification of mitral regurgitation, the complex left ventricular flow field and anatomy make it almost impossible to place a single slice such that it isolates the regurgitant flow (figure 1). In particular, the presence of the strong left ventricular ejection flows over the anterior mitral leaflet and the width of the left ventricle prevent this.[16] To combat these limitations a new method had to be considered. This has led to the development of the control volume theory to quantify mitral regurgitation.

The success of this new theory depends on obtaining a number of contiguous side by side velocity encoded MRI slices centered on the

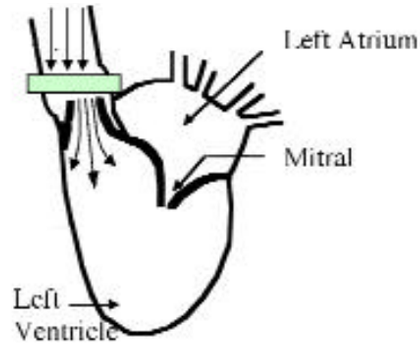

Figure 1A

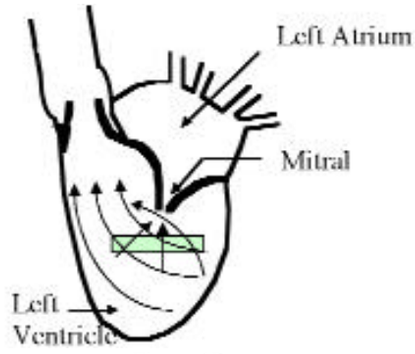

Figure 1B

Figure 1: Single Slice Method. The single slice method can be done to quantify aortic regurgitation (Fig. 1A), however cannot be used for mitral regurgitation because of the complex flow field (Fig. 1B). As one can see, flow going through the MRI slice is not guaranteed to contribute to the regurgitant flow thus invalidating the procedure.

mitral valve regurgitant orifice. This type of MRI allows the operator to measure all three velocity components (x, y, z) of fluid passing through those slices; the velocity of blood in the right-left direction (x-axis), anterior-posterior direction (y-axis), and in the foot-head direction (z-axis). A control surface is then drawn encompassing the mitral valve (figure 2).[17]

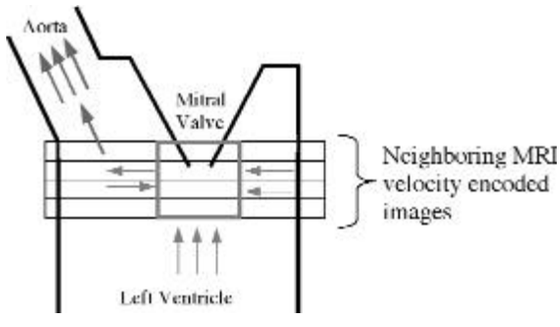

Figure 2: Blood flow (arrows) surrounding the mitral valve can be isolated by an imaginary control volume box (outlined) with the use of multiple MRI images.

Since the box will be constructed to cut the regurgitant orifice parallel to the orifice plane, there will be a ventricular side to the box and an atrial side.[15] Assuming fluid is incompressible and since matter cannot be created or destroyed, the principle of mass conservation states that the

inflow into this box is also equal to the outflow. Theoretically, the net volume of fluid which enters the control box during systole should be equal to the regurgitant volume given that its only exit is through the mitral valve. Furthermore, since the control surface is drawn such that it encloses only the regurgitant orifice, the ventricular outflow to the aorta will not be incorporated into the flow rate calculations.[15,16] The control volume method is therefore robust, appropriate and probably necessary for the quantification of mitral regurgitation.

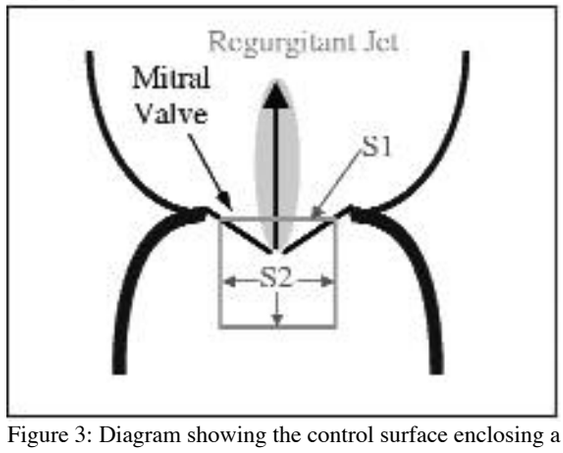

Figure 3: Diagram showing the control surface enclosing a regurgitant mitral valve. S1 = the part of the control surface on the atrial side of the mitral valve; S2 = the part of the control surface on the ventricular side of the mitral valve.

We can deduce from figure 3 that the net flow going into the ventricular side of the control box must be equal to the regurgitant flow on the atrial side (S1). With the MRI images supplying the velocity of fluid, the flow rate of fluid crossing the boundaries can be calculated by the product of the normal velocity x area. An easier tactic may be to calculate the atrial side (S1) directly; however, because of the difficulties in obtaining accurate velocity measurements in the turbulent regurgitant jet, this approach is not possible. Therefore in order to quantify the regurgitant flow, the term S2 must be evaluated.[16] This area is an imaginary three-dimensional cube and in order to obtain the flow rate through one side of the cube (the side which contains the regurgitant jet), the flow rate through the other five sides must be determined. Summing the flow rate for the five sides will then give a net total flow rate, which is the negative equivalent of the flow rate going through the sixth (atrial) side. By obtaining the net flow rates going into the ventricular side of the CV box over time, the total regurgitant volume can then be determined by integration of the flow rates with respect to time over the systolic phase.[16]

WHY USE MRI?

Magnetic resonance imaging is an attractive tool for evaluation of patients with various heart diseases. First, it is safe and easily performed in an outpatient setting without the need for intravenous injections or the use of ionizing radiation.[8] Second, it provides cardiovascular flow information rapidly and efficiently. Because of its ability to produce images relatively inexpensively and also non-invasively, it allows repetitive evaluations of the left ventricular and regurgitant volume measurements to be made in a safe manner. Also the ability of MRI to obtain tomographic images in virtually any plane gives it a tremendous advantage over other imaging techniques. Its capability to acquire blood flow velocity information in all three dimensions within a single slice also provides MRI with an advantage over conventional imaging techniques.[18] This particular feature is especially important as it is the limiting factor for the other quantifying techniques when calculating the flow rate. It is necessary that only the velocity perpendicular to the surface be integrated since the other velocity components do not pass through the control volume surface. They therefore cannot contribute to the flow out of or into the control volume.[15] Doppler ultrasound, for example, can be used only to measure a single velocity component and thus, it cannot be used to measure the velocity normal to each surface.[12] As this is needed to calculate the true flow rate, the ultrasound method will result in calculating an inaccurate flow rate. MRI is superior in this respect. The velocity is measured in each pixel of the MRI image and each velocity component is measured individually so that the vertical, horizontal, and through-plane velocities are separate images.[17] In order to account for cardiac motion and the pulsatility of the flow, a number of MR images are obtained per heartbeat. These are approximately 20 to 40 milliseconds apart and are synchronized with the heartbeat by triggering the acquisition of data from the patient's electrocardiography (ECG).[12] Lastly, by using data obtained from MRI, the need for an assumed shape for the control volume is removed. Any shaped control volume could be used because the velocity component normal to the control volume surface can always be calculated from the three separate velocity components. To simplify the calculation however, a rectangular control volume is used.

CONCLUSION

In the clinical decision making process regarding mitral regurgitation, accurate determination of the severity of the disease is of major importance. The risk of surgery warrants confirmation of severity by a complementary

method. At present however, mitral regurgitation has no gold standard against which to quantify the regurgitant volume.[6] In addition, the effects of medication to treat symptoms and underlying conditions associated with mitral valve regurgitation cannot be studied in more detail as no quantification technique today can monitor the relative changes of the regurgitation process accurately without some type of invasive procedure.[5,6] This paper explores a new MRI technique that could in theory accurately and safely quantify mitral regurgitation using a control volume method. The concepts behind the technique begin with taking a number of neighboring imaging slices in the vicinity of the mitral orifice and measuring all three-dimensional velocity components via MR velocity encoded imaging. An imaginary three dimensional control volume box is then constructed to encompass the mitral valve. True to the principle of mass conservation, the calculated net inflow into the imaginary box from the ventricular side is equal to the regurgitant flow through the orifice.[16] No studies yet describe the testing of this technique in-vivo, however, studies from in-vitro flow phantom have shown excellent results in terms of precision.[15,16] With further work, this technique has the possibility of ending the search for an accurate non-invasive method to quantify mitral regurgitation.

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A Comparative Dataset of Six Cancers in the European Union

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INTRODUCTION

Cancer causes one-quarter of all deaths in Ireland and is the largest single cause of death for the Irish population. In Ireland in 1999 patients with cancer accounted for over 300,000 in-patient days in hospital, thus making the disease a major drain on the economy.[1] A similar situation exists throughout the European Union (EU). In order to fight what has become the biggest health concern in Europe, it is necessary to examine all the available data.

Cancer registries are becoming more common and extremely valuable in the information that they provide. The European Network of Cancer Registries produces EUCAN a database which pools all the cancer statistics from the EU countries.[2] EUCAN is a unique source of the most up to date information on cancer incidence, mortality, prevalence and survival in the EU and its member states. A database such as this allows for comparisons with other pooled data sources, such as those held by the Organisation for Economic Co-operation and Development (OECD), enabling us to gain a comparative view of cancer costs. While the information provided is invaluable, there is still a need for standardisation of recording methods. For example, Italy has 14 registries each with its own separate standard of measurement. In spite of this, pooled databases cast light on discrepancies and similarities between people that would be considered to have similar genetic and cultural backgrounds.

In terms of focus, the current study looks at six cancers listed in the database, which are diverse in their treatment, screening and aetiology: oesophageal cancer, colorectal cancer, lung cancer, prostate cancer, breast cancer, and cancer of the cervix.

Oesophageal Cancer

The aetiology of oesophageal cancer is very complex. Smoking and excessive alcohol consumption, especially of spirits, are thought to be major risk factors. While they are independently significant, the risk of developing oesophageal cancer is more than 100 times greater when the two are combined.[3] A higher incidence is also associated with diets low in vegetables and fruit and deficient in vitamins or trace elements.[4] Plummer-Vinson syndrome, long-standing achalasia and Barrett's oesophagus all increase an individual's risk. The only hereditary transmission of oesophageal cancer is in patients with a genetic condition known as tylosis.[8] Incidence increases with advancing age. Oesophageal cancer is rare below the age of 40 and peak incidence is between 60 and 70 years of age.[4]

Oesophageal cancer can exist *in situ* for up to three or four years. The disease is largely asymptomatic in its early stages, and thus usually presents at an advanced stage. At the moment, the only way to screen for oesophageal cancer is endoscopy with biopsy.[5] Screening for oesophageal cancer is not widely available as there are high risks associated with these procedures such as perforation of the oesophagus. Screening is only recommended for high-risk individuals to provide early diagnosis in the asymptomatic patient.

Colorectal Cancer

Colorectal cancer is one of the major malignancies affecting western societies in terms of both incidence and mortality. Globally, risk seems to be correlated with economic development, with the highest rates in the western world and the lowest rates in Africa, South America and Asia.[6] This may be in large part due to dietary factors. Experimental evidence suggests that high dietary levels of meats, fats and refined carbohydrates promote colorectal cancer.[7] As most of the countries included in our study are considerably industrialised and have a high *per capita* income, a high incidence of colorectal cancer might be expected. A positive family history for colorectal cancer is another important risk factor.[7] A number of conditions such as ulcerative colitis and familial adenomatous polyposis coli predispose to the development of the disease.[8]

The incidence of colorectal cancer in the European Union, and the western world in general, is sufficiently high for screening to be a realistic proposition. There are three methods: faecal occult blood testing (FOB testing), digital rectal examination (DRE) and sigmoidoscopy. These investigations are relatively inexpensive; however FOB testing has a high level of false positives and DRE lacks sensitivity. Most cases of colorectal cancer occur in patients over 50 years

old, so a high-risk group is easy to identify.[8] A new technique known as virtual colonoscopy is proving to be the way forward in screening for this disease. This technique uses a reconstructed image based on Computed Tomography (CT) data.

Lung Cancer

The majority of cases of this disease are attributed to exposure of the bronchial epithelia to inhaled carcinogens.[9,10] There is a strong causal relationship between cigarette smoking and lung cancer with approximately 95% of cases associated with tobacco.[6] Risk is related to duration and intensity of smoking.[9] Other factors include exposure to asbestos, radon gas, air pollution and passive smoking.[10] Lung cancer had always been more common in men than in women, though recently the incidence in women has been increasing due to the increase in smoking among women.[11]

There are two different forms of lung cancer: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC grows rapidly and is likely to spread to other organs. Patients can die two to four months after development if not treated. NSCLC is a silent disease and grows very slowly, sometimes going unnoticed for years.[3] In the past, screening was shown to be ineffective, with no improvement in patient care or survival rates.[10] However recently at an International Lung Cancer Conference recommendations were made for spiral CT screening of the chest for high-risk patients to improve survival rates. The normal survival rate for lung cancer is 10% but with this screening test survival jumps to 70% for very early stage lung cancer.[12]

Prostate Cancer

Carcinoma of the prostate is a disease of ageing men, with a peak incidence and mortality of approximately 70 years of age.[13] The disease is rare under the age of 45 years, and is almost universal at post-mortem in men over 80 years of age.[14] Even taking into consideration the limited population affected by this disease, it is one of the most common forms of cancer in men.[8] The disease is more common in married men and is thought to be related to the number of sexual partners, frequency of sexual activity and a history of a sexually transmitted disease.[14]

The main methods available for prostate cancer screening are digital rectal examination and prostate-specific antigen (PSA) testing. Both of these are problematic as there is a chance of obtaining false negatives with PSA tests and digital rectal examination lacks sensitivity.[13] At present there is much debate over the value of prostate screening and whether it is ethically correct. Although prostate cancer is extremely prevalent in men over 80 it is mostly asymptomatic, which means that many men may never realise that they have the cancer and die from other causes.[15] If a screening program were introduced to identify prostate cancer, it would result in many more men living with the diagnosis of cancer, and undergoing treatment which may not be of benefit in terms of reducing disease-related morbidity or mortality. Treatment-related morbidity would be increased with no benefit. Prostate screening would therefore not fulfil Wilson and Junger's criteria for implementation of a screening program: that the chance of harm is less than the chance of benefit.[15] Quoted from Health Technology Assessment, "evidence concerning effectiveness of screening in reducing the number of prostate cancer deaths is very poor. There is no justification for the introduction of population screening."[15] This is a serious ethical issue and further studies must be taken before population screening for prostate cancer could be implemented.

Breast Cancer

Breast cancer is the most common malignant disease in women.[8] Up to 8% may be due to inherited genetic abnormalities.[16] Relative risk is increased three-fold when primary relatives have been affected.[8] History of benign breast disease, early menarche, late menopause, use of oral contraceptives, hormone replacement therapy and obesity increase an individual's risk of developing the disease.[17]

There is sufficient evidence to suggest that screening women aged 50 to 69 years is effective. A one-third reduction in mortality from breast cancer among these women has been demonstrated. There is also limited evidence showing some efficacy in women aged 40 to 49 years. Screening is carried out by means of mammography with or without physical examination of the breasts, and follow up of positive suspicious findings is by biopsy. National screening programmes have expanded rapidly in Western Europe.[18] Women are also advised to carry out 'self-checking' as this is often how the malignancy is first detected.

Cancer of the Cervix

Cervical cancer is strongly associated with the human papilloma virus. Young age at first sexual intercourse, multiple sexual partners, promiscuous male partners, cervical dysplasia and smoking increase the risk of developing the disease.[8] Risk decreases with the use of the contraceptive pill and number of full-term pregnancies.[19]

Cervical cancer has a long developmental phase with a detectable pre-clinical phase, which makes it ideal for screening. Screening for cervical cancer has been shown to reduce mortality. It is carried out by means of a Papanicolaou (Pap) smear test. This cytological test is far more effective in reducing mortality from cervical cancer than mammography screening in breast cancer. This test is widely available to women over 25 years of age in many European countries and it is recommended that the test be repeated every 3 to 5 years, although a screening program with an interval of 10 years can reduce the incidence of invasive cervical carcinoma.

METHODS

The data used in this paper was obtained from the EUCAN Database.[20] This website contains a breakdown of the incidences (including crude rate and age specific rate for Europe), mortality (including crude rate and age specific rate for Europe), 1-year prevalence and 5-year prevalence for various different sites of cancer in different European populations. The data on this website represented cancer statistics for all European Union Countries for the year 1998.

STATISTICS

Incidence is the number of new cancer cases arising in a given period of time in a specific population. Mortality is the number of cancer deaths occurring in a given period of time in a specific population. The age-standardised rate (ASR) is a summary measure of a rate that a population would have if it had a standard age structure. The figure used is per 100,000 people in the population. The ASR is important when comparing several populations that differ with respect to age, as one is comparing like with like and removing a bias, so making the comparison more accurate. This is especially important when investigating the incidence of cancer, as it is an age-related disease.

The mortality:incidence (M:I) ratio is the expression of newly reported cases to reported deaths, giving an indication of survival. As part of our calculations, we took the incidence as 1 and expressed the mortality as a fraction of that.

RESULTS

Table 1 demonstrates mortality:incidence ratios for the six selected cancer sites in 15 EU countries compared to the EU average. The figures below are calculated as the mortality if the incidence is taken as 1.

Table 2 shows the rank order of incidence of the six selected cancer sites in 15 EU countries with comparison to the EU average. The figures that are used in the table below are age standardised rates. They are taken per 100,000

Table 1: Mortality:Incidence for the Six Selected Cancer Sites in 15 EU Countries

	Oesophagus	Colorectal	Lung	Breast	Cervix	Prostate
Country	**Both***	**Both***	**Both***	**Female***	**Female***	**Male***
EU	**0.90**	**0.49**	**0.91**	**0.31**	**0.39**	**0.38**
Austria	0.84	0.47	0.93	0.32	0.36	0.30
Belgium	0.92	0.48	0.91	0.32	0.43	0.32
Denmark	1.07	0.56	0.95	0.33	0.40	0.55
Finland	0.84	0.45	0.94	0.23	0.32	0.25
France	0.86	0.46	0.92	0.26	0.35	0.31
Germany	0.86	0.52	0.91	0.32	0.40	0.38
Greece	0.89	0.47	0.89	0.31	0.39	0.42
Ireland	0.89	0.46	0.99	0.35	0.40	0.44
Italy	0.96	0.45	0.89	0.30	0.34	0.36
Luxembourg	0.92	0.52	0.93	0.24	0.42	0.31
Netherlands	0.92	0.46	0.94	0.32	0.36	0.35
Portugal	0.95	0.47	0.90	0.33	0.41	0.51
Spain	0.94	0.49	0.88	0.33	0.41	0.53
Sweden	0.91	0.45	1.01	0.22	0.36	0.33
United Kingdom	0.93	0.51	0.90	0.35	0.44	0.43

people in the affected population and adjusted for age.

Table 2: Rank Order of Incidence of the Six Selected Cancer Sites in 15 EU Countries.

Country	Oesophagus	*Country*	Colorectal	*Country*	Lung
Greece	1.4	Greece	24.19	Sweden	23.69
Italy	2.8	Finland	32.46	Portugal	25.68
Sweden	3.09	Sweden	39.71	Finland	32.22
Finland	3.23	UK	41.74	Austria	36.66
Austria	3.55	Spain	41.77	Germany	39.03
Spain	4.2	Italy	43.53	Spain	39.57
Portugal	4.31	France	43.68	France	39.68
Germany	4.53	***EU***	***44.04***	***EU***	***42.16***
Belgium	4.86	Portugal	44.37	Ireland	43
Denmark	5.15	Belgium	45.74	Italy	43.79
EU	***5.38***	Luxembourg	48.73	Greece	44
Netherlands	6.29	Germany	48.74	Luxembourg	46.03
Luxembourg	6.6	Austria	49.19	UK	50.56
France	7.61	Netherlands	49.85	Netherlands	51.96
Ireland	9.04	Ireland	50.86	Denmark	52.75
UK	9.43	Denmark	51.8	Belgium	55.15
Country	**Breast**	***Country***	**Cervix**	***Country***	**Prostate**
Spain	66.81	Finland	5.6	Greece	41
Greece	67.97	Luxembourg	7.53	Spain	45.33
Portugal	70.46	Spain	7.55	Italy	52.78
Austria	86.14	Greece	8.14	Denmark	53.89
Italy	87.87	Netherlands	8.22	Portugal	55.23
Luxembourg	89.24	Italy	8.43	UK	60.97
Germany	89.43	Sweden	9.23	***EU***	***67.55***
Ireland	91.01	UK	9.45	Ireland	69.57
EU	***92.04***	***EU***	***10.3***	Germany	70.21
UK	94.66	Belgium	10.66	Luxembourg	78.53
Finland	102.02	Ireland	11.75	Netherlands	85.74
France	107.7	Germany	12.07	France	87.1
Netherlands	112.04	France	12.41	Austria	89.49
Sweden	113.98	Portugal	12.89	Belgium	95.34
Belgium	114.27	Austria	13.25	Sweden	114.95
Denmark	115.45	Denmark	14.47	Finland	121.84

The incidence of oesophageal cancer in selected EU countries is shown in figure 1. The error bars represent 1 standard error of the mean values within the EU.

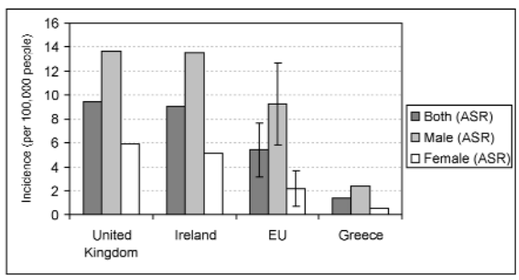

Figure 1: Incidence of Oesophageal Cancer in Selected EU Countries.

The United Kingdom presents with the highest incidence of oesophageal cancer for both sexes combined as well as males and females alone. In comparison, Greece presents with the lowest incidence in all three categories. The incidence in Ireland is also quite high, almost equaling that of the UK.

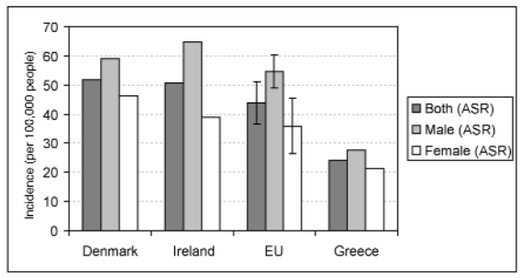

Figure 2: Incidence of Colorectal Cancer in Selected EU Countries.

As seen in figure 2, Denmark has the highest incidence in colorectal cancer overall with Ireland following closely behind. However, Ireland has the highest incidence in males. Greece has the lowest incidence in all three categories. The error bars represent 1 standard error of the mean values within the EU.

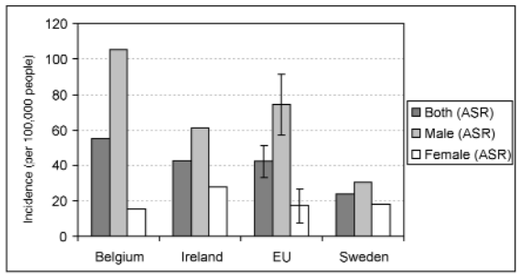

Figure 3: Incidence of Lung Cancer in Selected EU Countries.

Figure 3 shows that Belgium has the highest overall incidence of lung cancer, but it has the lowest incidence of lung cancer in females. Sweden has the lowest overall incidence. The error bars represent 1 standard error of the mean values within the EU.

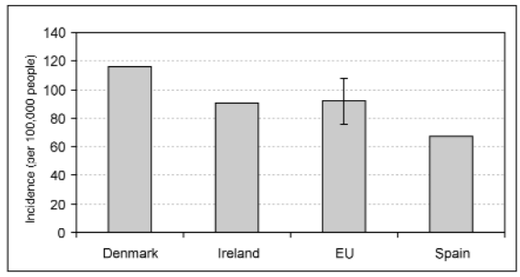

Figure 4: Incidence of Breast Cancer (Female only) in Selected EU Countries.

Figure 4 clearly demonstrates that Denmark has the highest incidence of breast cancer whereas Spain has the lowest incidence. The error bar represents 1 standard error of the mean values within the EU.

As seen in figure 5, Denmark has the highest incidence of cancer of the cervix whereas Finland has the lowest incidence. The error bar represents 1 standard error of the mean values within the EU.

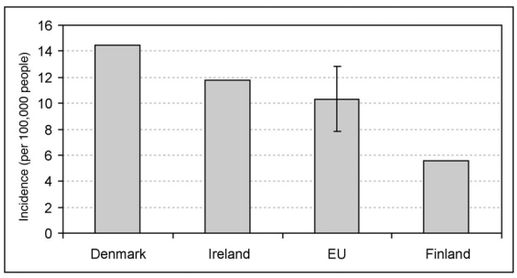

Figure 5: Incidence of Cancer of the Cervix(Female only) in Selected EU Countries.

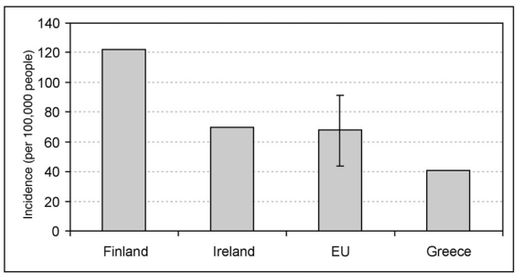

Figure 6: Incidence of Prostate Cancer (Male only) in Selected EU Countries.

Figure 6 clearly shows that the highest incidence of prostate cancer is in Finland and is almost double that of the EU average. The Irish incidence is very close to the EU average. Greece has the lowest incidence. The error bar represents 1 standard error of the mean values within the EU.

DISCUSSION

Oesophageal Cancer

The countries which presented with the highest incidence of oesophageal cancer were Ireland and the UK, while Greece had the lowest incidence rates (table 2). The differences between male and female incidence rates for oesophageal cancer are significant for all populations studied. This is likely to be due to the social culture present in the UK and Ireland, which expose the male population to regular alcohol intake and tobacco smoke, the two main risk factors in oesophageal cancer. In the aetiology of oesophageal cancer, tobacco smoke and alcohol act synergistically. The relative risk of an individual who both smokes and drinks is much greater than that of the individual who either smokes or drinks.[3]

In relation to the M:I for oesophageal cancer, the proportion of those who die from this form of cancer is high. Denmark and Italy have the highest M:I ratio for both sexes. Finland has the lowest M:I ratio. The M:I ratio for both sexes is large. There is quite a difference between this ratio in Belgium with a male M:I ratio of 0.91 and a much lower female M:I ratio of 0.46 (table 1). In contrast however, the male and female M:I ratios of Denmark are both similar and higher than the European average.

Oesophageal cancer has the highest M:I ratio in Denmark and this may be directly related to the number of cigarettes smoked and the high amount of alcohol consumed. For example, during the seventies and eighties a dynamic change in drinking habits and beverage choice took place in Denmark. *Per capita*, wine consumption rose from 5.91 litres in 1970 to 21.31 litres in 1990, an increase of 260%. In the same time, the absolute consumption of alcohol also rose.[21]

Colorectal Cancer

Ireland has one of the highest incidences of colorectal cancer (table 2). Ireland's M:I ratio of 0.46 is lower than that of the EU so although the incidence is high, people are surviving the disease (table 1). However, the Irish diet contains too much fat and too little carbohydrate and this is a factor in the high incidence of the disease.[22] Greece has the lowest incidence, which is likely to be related to their Mediterranean diet of low saturated fat.

It is also very interesting to see that Denmark has the highest M:I ratio and incidence (above the EU average). The Danish diet is high in fat and this could contribute to the incidence.[23] This may suggest a need for public health campaigns and the high incidence may make screening a viable option. The treatment techniques should also be reviewed in view of the M:I ratio. Non-starch polysaccharides (fibre) and vegetables are established factors that reduce risk largely owing to the effect of non-starch polysaccharides in regulating bowel function. An intake of 18g per day of non-starch polysaccharides is recommended. This increases stool weight and reduces constipation thus reducing the risk of bowel cancer. Eating greater amounts of red meat also increases an individual's risk, as there is an association between metabolism of meat and the risk of developing polyps, which can become cancerous. Meat also increases the amount and type of residue entering the bowel, thus affecting the by-products formed by the colonic flora. These by-products, which include N-nitroso compounds, may be carcinogenic.[24]

Lung Cancer

Belgium presents with the highest incidence of lung cancer in Europe, whereas Sweden has the lowest incidence of lung cancer (table 2). Similar to oesophageal cancer, there are visible differences between the incidences in males and females. An example of this is in Ireland where the ratio of male to female lung

cancer incidence is almost 2:1 (figure 3). The M:I ratio in Ireland is also high for lung cancer (table 1).

Sweden has the highest M:I ratio at 1.01 (table 1) which means that more people died from lung cancer than were diagnosed in those years. Once again the M:I in Denmark is well above the EU average. Of note also is that Ireland is 2nd only to Sweden in terms of the worst survival from lung cancer. Spain has the lowest M:I ratio for both sexes. There is not much variation between the male and female incidence for this country.

There is a clear dose-response relationship between lung cancer risk and the number of cigarettes smoked per day, the degree of inhalation and the age of initiation of smoking.[9] The rate of smoking in Ireland, Denmark and the UK are high and therefore these countries present with high incidence rates. One of the most remarkable finding from Denmark is that it presents with the highest pre-standardised rates for female lung cancer in the world. In the beginning of the 1950's, the prevalence of smoking among Danish females was as high as 40% and in the 1980's; the proportion of females who smoked was almost the same as males.[25] This in turn explains the substantial number of females presenting with and dying from lung cancer in Denmark.

Another reason for the high rates of cancer in Denmark is the fact that radon gas is related to 10% of all lung cancers, accounting for 300 deaths per year.[26] The high level of radon gas is due to geographical and geological reasons. The European average for radon-caused cancers is five per cent, while in Denmark, this is doubled.

Breast Cancer

Our results show that Denmark has the highest incidence of breast cancer in the EU. A high incidence of breast cancer may indirectly reflect a good national screening programme. High incidence of breast cancer is due to genetic factors, and long-term levels of hormones in the blood.[17] It is very hard to attribute breast cancer to a single risk factor, as it is known that multiple factors are to blame. One contributing factor could be that Danish women have a poor diet that is high in fat. They have the highest rate of smoking amongst women in Europe and this could attribute to the high incidence. Incidence of breast cancer in Ireland is similar to the EU average, at an ASR of 92.04.

With regard to the M:I ratio, Ireland ranked the highest in Europe (table 1). The figures show that over 35% of women diagnosed with breast cancer in Ireland die from the disease. This suggests that screening in Ireland at this time was insufficient and the need for a national screening programme was extremely important. Also treatment techniques and waiting times for treatment may need to be examined. However these results were taken from 1998 which is the same year that the first phase of the programme was set up. Screening did not actually begin until 2000 and therefore any results yielded from this programme would not be reflected in statistics until subsequent studies were carried out post 2000. However, it must be noted that although the highest in the EU, the average figure is over 30%. Sweden has the lowest M:I ratio, which may suggest that many women are diagnosed at an early stage.

Cervical Cancer

As demonstrated in table 2, there is an approximate three-fold increase in incidence of cervical cancer between the countries that ranked the lowest and highest. Even though the absolute values are relatively low, this is quite a large gap.

Finland has the lowest incidence (table 2) and M:I ratio (table 1). Since the 1960s, Finland has had a national cervical cancer-screening programme in place, which would decrease the incidence.[27] Normally screening would increase the incidence but as the test detects the disease in a pre-malignant stage, the progression of cells to full malignancy is reduced. Figure 5 demonstrates that there is a large variation in the incidence of cervical cancer and this may be attributed to screening. Another reason for the low incidence of cervical cancer in Finnish women may be that they have one of the lowest smoking rates among women in Europe.[28] As smoking is seen as one of the risk factors for cervical cancer, this may be a supplementary factor.

Denmark has the highest ASR incidence (table 2). Public awareness campaigns may be necessary. As smoking plays a part in the aetiology of this disease and alcohol consumption is very high in Denmark, a partial explanation could lie here. Although Denmark has the highest incidence of cervical cancer it ranks sixth highest in Europe for M:I ratio. The U.K has the highest M:I ratio in Europe (table 1), which indicates that they must take a closer look at their treatment practices and the use of Pap smear tests in a screening programme to detect the disease at an earlier stage to decrease the mortality rate. Ireland also has a larger M:I ratio than the EU average and the same recommendations must be made here. A new national screening programme is due to be in place in Ireland in the near future.

Prostate Cancer

Finland has the highest incidence of prostate cancer while Greece has the lowest (table 2). The high incidence in Finland is possibly due to screening. In Finland in 1996/97, a nationwide study was carried out on prostate cancer. More men were screened and more made aware of their condition. Greece does not have well developed screening implying that patients are not diagnosed, even though the condition may be present. Finland's M:I ratio is the lowest ratio in the EU at 0.25 (table 1). This shows that although there is a high incidence, there is a very low death rate. This can be interpreted as proof that screening programs can be effective. By comparison, in Greece the M:I is above the EU average. However in the case of prostate cancer which is often asymptomatic and may not be the cause of death, we would suggest caution at comparing M:I data between countries that have screening programs and those that do not.

Although Greece had very low figures in incidence and M:I ratio for all the selected cancer sites, the results come from the southern Italy registry. Therefore the registries are insufficient and may not provide a true reflection of the cancer situation in Greece.

CONCLUSION

Throughout all the cancer sites that were studied in this paper, Denmark produced alarmingly high results in both incidence and M:I ratio for specific cancers. The figures for the latter are more worrying as they reflect the number of people who are dying from cancer. With cancer, no one factor can be pinpointed as the cause of disease. During research for this paper factors were found that may be a cause for this high incidence and mortality in Denmark. These include the high saturated fat in their diet, high alcohol consumption and high level of tobacco intake.[21] What is most striking is that Denmark departs from the Nordic countries in its incidence and outcomes of cancer. This illustrates that the genetic and cultural similarities are outweighed by lifestyle choices at a national level. Furthermore, the public awareness of cancer in Denmark is arguably lower than that of their Nordic neighbours. This is surprising, given that the educational systems of all four countries are reasonably similar. The divergence would appear to be at a governmental level, where health services planning and health promotion activities are coordinated.

Incidence of cancer in Ireland is above the EU average in every site studied except for breast cancer. The most worrying cases are those of oesophageal and colorectal cancer which have a much higher incidence in Ireland compared to the EU average. This shows that cancer is a major concern to Irish health services and that both public health campaigns and research are vital.

It is likely that there will be further refinements in cancer registries as clinicians, governments, and health insurers will want to have more information on cost effectiveness in order to better shape cancer strategy. As governments are establishing their cancer management plans, they need to have an overall view of the epidemiological state of their country in order to effectively distribute available funding. For example, if the government had to pay to treat a Stage IV lung cancer it would cost the state approximately 7,500.[29] However, if the government invests in cancer awareness campaigns, the future cost may decrease as the incidence decreases.

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How might Operative Dentistry be a Threat to the Pulp?

Reshma Seeburrun

INTRODUCTION

Operative dentistry is the art and science of diagnosis, treatment and prognosis of defects of teeth that do not require full coverage restorations for correction. Such treatment results in restoration of proper tooth form, function and aesthetics while maintaining physiologic integrity of the adjacent teeth and soft tissues, all of which should enhance the general health of the patient.[1] Examples of such procedures include simple amalgam fillings, inlays, onlays and preliminary periodontal surgery. The pulp is a viscous connective tissue of collagen fibres and ground substance supporting the vital cellular, vascular and nerve structures of the tooth (see figure 1). Pulpal responses to dental treatment depend on many factors including thermal injury, injury to odontoblastic processes, desiccation of dentin, vibration, pulp exposure, smear layer, remaining dentin thickness, restorative materials used and microleakage.

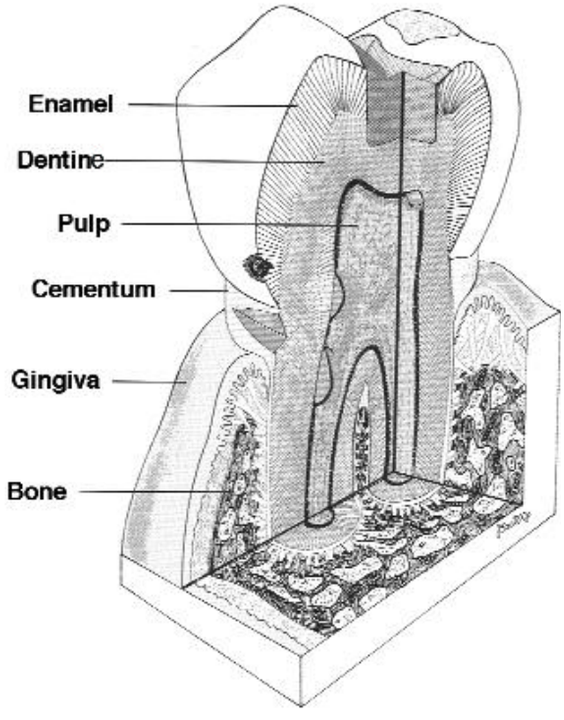

Figure 1.[6]

THERMAL INJURY

Tooth preparation with a rotating bur or stone produces a considerable amount of frictional heat. The amount of intrapulpal heat generated is determined by many factors, including the drill rotation speed, size, type and shape of cutting instrument, length of time the instrument is in contact with dentine, the amount of pressure exerted on the handpiece, the cutting technique and the use of coolants.

Studies have shown that high-speed cutting with copious water coolant and a reduced force results in minimal histological alteration of the pulp.[2] In fact, Stanley showed in 1976 that given the same conditions of comparable remaining dentine thickness (RDT) similar cutting instrument and adequate water cooling, the intensity of the pulpal response will be less with high speed rather than lower speed cutting. This is due to the lower application force required by the high speed handpiece.[3] Wittrock *et al*.[4] have shown that the cutting technique also greatly influences heat generation. It is not advisable to start removing amalgam by making a deep internal slot within the restoration as this results in the localised concentration of heat. Preparation for restoration close to the pulp may generate substantial frictional heat causing a significant and detrimental temperature increase in the pulp. Repair will usually occur but the formation of reparative dentine can be extensive and render the pulp vulnerable to repeated injury. In fact, clinical follow-ups of teeth restored with cast restorations (full crowns and teeth included as abutments in bridgework) have shown that pulpal necrosis may occur with a frequency of 10-15% over a period of 5-10 years.[5] Often one will find that the coronal portion of the pulp in such teeth is obliterated by reparative dentine, making endodontic therapy precarious.

Another complication to cavity and crown preparation is internal bleeding. In rare cases it may be so extensive that pulpal necrosis occurs almost instantaneously. The tooth structure of such teeth may turn red and later a grey colour.

INJURY TO ODONTOBLASTS

Odontoblasts are exposed to a variety of insults, including frictional heat, amputation of processes, displacement of the cell body, vibration and exposure to bacterial toxins and other chemical irritants. The odontoblastic cells are packed closely together with both permanent and temporary junctions between the cellular membranes. The integrity and spacing of the odontoblastic layer mediates the passage of molecules and tissue fluids between the pulp and dentine. Routine dental procedures can

temporarily disrupt the odontoblastic layer and may sometimes inflict permanent cellular damage.[7] Depending on the depth of the cavity preparation, odontoblastic processes are amputated at various points along their distal segment. If the processes are not amputated close to the cell body then repair of the cell membrane occurs. However if the odontoblasts do die they undergo autolysis and are replaced by new cells derived from odontogenitor cells. This replacement occurs provided that the underlying cell-rich zone of the pulp has not been injured.[8]

DESSICATION OF DENTINE

The use of compressed air to dry a cavity preparation for a prolonged period can result in a delayed healing response. Drying surface dentinal fluids activates strong capillary forces which cause a rapid outflow of fluid in the dentinal tubules. This rapid outflow stimulates mechanoreceptors, which not only cause post-operative pain, but can even cause the displacement of the odontoblast from the odontoblastic layer up the tubule. The displaced cells undergo autolysis and disappear. These can be replaced with cells from the uninjured underlying pulp. Contrary to popular belief, vigorous drying alone does not cause major irreversible pulpal reactions.[9] This is because the products of degradation are so diluted by the dentinal fluid as to induce no inflammation in the pulp. Also, it could be due to the fact that too few cells are involved to cause a significant reaction. Tertiary dentine or reparative dentine is then laid down in about 1 to 3 months by the odontoblasts to wall off the pulp from the site of injury.

VIBRATION

The effects of the vibratory movement of cutting burrs on the pulp have not been thoroughly researched. According to Holden the shock waves generated occur beneath the point of application of the bur in the pulp.[10] They appear to be more pronounced when the bur is stalled by digital pressure. Stalling not only decreases cutting efficiency by clogging up but also leads to an increase in temperature.[2]

PULP EXPOSURE

Exposure occurs most often in the process of removing deep carious dentine. Accidental mechanical exposure may result during placement of pins and retention points in dentine. Friction-locked pins often produce microfractures, which establish communication between the pulp and dentine. In all these cases the pulp appears to be affected primarily by bacterial contamination. Frank showed that exposure of the pulps in germ-free rats was followed by complete healing without any inflammatory reaction.[11] No such published studies were found in the review of the literature.[11]

SMEAR LAYER

The use of rotary instruments leaves an amorphous layer of microcrystalline debris on the enamel and dentine surfaces, known as the smear layer. Some controversy exists regarding the value of removing or maintaining this layer. One school of thought purports that the debris occludes the dentinal tubules, decreasing dentine permeability and preventing the ingress of bacteria.[12] However, Pashley suggested that this does not prevent the ingress of bacterial toxins and by-products that can lead to pulpal inflammation.[13] Moreover, Brannström believes that most restorative materials do not adhere to dentinal walls and bacteria from the smear layer may invade the contraction gaps.[14]

MICROLEAKAGE

In spite of substantial efforts over the years to improve the composition of restorative materials, including resin composites and the techniques for their use, the shrinkage of these materials after setting is critical.[15,16] Shrinkage builds up strains in the filling that later may result in gaps at the interface between tooth and restoration. This may allow bacteria and bacterial products in the oral environment to affect the pulp. The term microleakage is used to imply this form of pulpal irritation.

Research in recent years has indeed demonstrated that bacterial leakage in restoration margins is a major threat to the vital functions of the pulp subsequent to restorative therapies.[5,17] In particular, on deep and extensive exposures of dentine, the infectious load on the pulp can be substantial. In principle, inflammation of the pulp in response to these bacterial exposures is similar to that for caries but there are some distinct differences. Neutrophils play an important role in the initial responses owing to the more sudden and extensive bacterial exposure than that in the relatively slowly progressing caries lesion. These cells accumulate in areas of the pulp that correspond to the involved dentinal tubules. Chemotactic stimuli also prompt neutrophils to migrate into the tubules. This is probably the most significant defence factor that, in addition to the protective effects of the dentinal fluid, helps to block further penetration of bacteria and bacterial elements into the pulp. Collectively these mechanisms are likely to explain why pulpal repair and healing are still possible even when a restoration does not completely seal the margins.

TOXIC EFFECTS OF RESTORATIVE MATERIAL

In addition to the trauma from preparing teeth for restoration and the subsequent leakage of bacterial elements, constituents of restorative materials may have an adverse influence on the pulp. For many years the toxicity of restorative materials was regarded as the major cause of adverse pulpal responses in restorative procedures. Some of the properties of restorative materials that are believed to cause or contribute to pulpal damage include chemical cytotoxicity, exothermic reaction on setting, acidity, absorption of water during setting and poor marginal adaptation leading to bacterial microleakage.

However, research in recent years has shown that, contrary to previous beliefs, toxic components in restorative materials are a lesser threat to the pulp than previously anticipated.[5] This has been best demonstrated in experimental studies where dental materials in common use, such as amalgam, zinc phosphate cement and resin composites, were applied directly on pulpal tissue in circumstances where the surface of the restoration was sealed bacterial-tight.[8,18] These experiments demonstrated that the pulp around the sealed restorations often resumed a healthy state. However without a bacterial-tight surface seal severe inflammation developed in the pulp. The risk of severe pulpal complication is even less when a dentine barrier remains. Dentine seems to serve as a detoxifying tissue in that highly toxic materials may be absorbed to the inner walls of the dentinal tubules. It has been shown that dentine buffers the effects of acids and bases.[19] Experiments in vitro and in vivo have demonstrated that catatonic components of resin monomers (e.g. triethylene glycol dimethacrylate (TEGDMA) and 2-hydroxyethyl methacrylate (HEMA)) readily penetrate thin dentine walls upon topical application.[20] The effect of such penetration is not well understood. However observations in animals suggest that the toxic effect on the pulp of this agent is short lasting. Furthermore, it has been shown that most leachable substances from resin composites are released within the first few days after placement and that then little will be discharged.[21] Therefore the threat to the pulp resulting from restorative procedures does not seem to be from the material *per se* but more from the improper seal that often results.

In addition, the exothermic reaction of some luting cements on setting has been thought to cause pulpal injury. However, Plant (1976) demonstrated that zinc phosphate, the most exothermic cement, caused an intrapulpal increase in temperature of just 2^{o}C.[22] This is insufficient to cause any injury to the pulp. Changing the amount of powder and liquid ratios when mixing cements however was shown to cause a marked rise in temperature.[23]

REMAINING DENTINE THICKNESS

Over the years, the degree of remaining dentine thickness (RDT) believed to be required in order to maintain a healthy pulp has greatly decreased. Stanley suggested that a RDT of 2mm is necessary to protect the pulp from most restorative procedures whereas Pameijer reported that a RDT of 1mm or more would protect the pulp tissue from the cytotoxic effect of zinc phosphate and resin-modified glass ionomer materials during the luting process.[24] Murray suggested that deeper cavities carefully cut down to 0.5mm appeared to have only a limited effect on underlying odontoblast survival.[25]

OTHER FACTORS

Root Planning

Pulp response to root planning is negligible unless dentine removal is excessive. Depending upon the remaining thickness of dentine the dentinal tubules are capable of repair and healing despite being exposed to microorganisms. However, if the apical foramen is involved periodontically, curettage could sever the blood vessels resulting in pulp damage. In this case these teeth are prophylactically root canal treated. It can be summarised that unless the root apices are involved, the effects of periodontal therapy on the pulp appears to be negligible.

Local Anaesthetics

Though intraligamentary injections have often been put forward as a cause for pulpal damage, clinical and animal studies have shown no adverse effects on the pulp.[26,27] Physiologic changes such as rapid and marked reduction in blood flow caused by adrenaline do occur.[28] However this vascular impairment has not shown any damaging effects on the pulp even in conjunction with restorative procedures.[29]

Electrosurgery

Electrosurgery, used in conjunction with operative dentistry to remove gingival tissue for enhanced access during tooth preparation and impression making, may affect the pulp. If the probe contacts a metallic restoration then adverse and often severe reactions occur.[30] These adverse reactions occur whether the restoration is based with a metallic or a non-metallic material.[31] The pulpal response is more severe with increased contact time and decreased RDT. Contact of more

than 0.4 sec has been shown to lead to irreversible damage.[32]

Bleaching of Vital Teeth

Vital teeth can be bleached using the "in-office" technique or the "at-home" technique. In-office techniques include the application of a bleaching agent, usually 35% hydrogen peroxide, to teeth isolated by a rubber dam. They may include activation of the hydrogen peroxide using heat or light in order to enhance or activate the release of peroxide. At-home bleaching uses a different bleaching agent, usually a 10% solution of carbamide peroxide, applied in a custom fitted tray that the patient wears at home, usually while sleeping. Many studies have shown that although penetration of the peroxide through the tooth to the pulp can produce sensitivity the pulp remains healthy and the sensitivity is completely reversible.[33,34] The rate of oxygen release, and therefore the rate of colour change, is proportional to the temperature. An increase of 10°C doubles the rate of chemical reaction.[33,35] However temperatures elevated to an uncomfortable level may result in tooth sensitivity or irreversible pulpal inflammation. Bleaching materials should always be administered without anaesthesia to avoid overheating the tooth.

CONCLUSION

Many operative procedures can be traumatic to the pulp and the effects are at least somewhat cumulative. The dental practitioner should be fully aware of the methods and materials that can jeopardise the pulp. Knowledge of proper cavity preparation and its application can greatly reduce pulpal injury. Many materials including cavity varnishes, liners and bases are widely available on the market to reduce the occurrence of pulpal damage. However other important factors that can determine a good pulpal prognosis are patient factors, which include age, previous treatment history, dental characteristics and diet.

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Pharmacological and Biological Circadian Therapy in Alzheimer's Disease

David Greaney
4th year Medicine

INTRODUCTION

Alzheimer's disease markedly affects the quality and quantity of both rapid eye movement (REM) and slow wave sleep (SWS).[1] The disease is a multi-factorial condition that also affects cognitive, behavioral and neurophysiological function. Accordingly, effective pharmacological treatment of Alzheimer's sleep related disorders focuses on achieving a balance in which the therapeutic benefits of improved sleep quality outweigh the adverse side effects.[1] Currently, no single pharmacological target has been implicated in the pathology in Alzheimer's type dementia (ATD) sleep disorders.[2] As the etiology of Alzheimer's disease (AD) still eludes science, pharmacologists are choosing to target the sleep-wake circadian cycle directly.[3]

Alzheimer's disease is a neurodegenerative process affecting pyramidal neurons with concurrent formation of "plaques and tangles" resulting from abnormal ß-amyloid protein production. Particularly affected are the cholinergic neurons found in the basal nucleus of Meynert and their extensive projections.[4] The characteristic "forgetfulness" (anteriograde amnesia) is derived from a decrease in hippocampal function.[5] Because the disease develops differently from person to person, researchers believe that there may be more than one pathologic process that leads to the same outcome.[6]

There are two main areas of the brain involved in sleep and both undergo degeneration in AD (Figure 1):

1- The Brainstem: Serotonergic and noradrenergic systems are active during the wakeful state and their activity declines markedly during REM sleep. Cholinergic neurons are active during REM sleep.

2- The Suprachiasmatic nucleus: receives direct input from the optic nerve and pineal gland.[7]

Acetylcholine (ACh) release from the basal ganglia oscillates daily and plays an essential regulatory role in the sleep-wake cycle. Furthermore, it acts as the main neurotransmitter in attention and learning. Because these neurons degenerate in AD, normal ACh levels regulating the sleep-wake cycle are theoretically vulnerable to cholinomimetic drugs. ACh release measured with the use of neostigmine changes significantly across the sleep cycle in cortex and hippocampus, substantia innominata, thalamus and medullary reticular formation.[8] Since ACh evidently plays an integral role in many brain processes, isolation of the cholinergic system with regards to sleep is a challenging prospect. Treatment of AD requires a pharmacological agent that targets only the degenerative cholinergic neurons; likewise, AD related sleep medications should only target the neurons pertinent to the sleep-wake cycle.

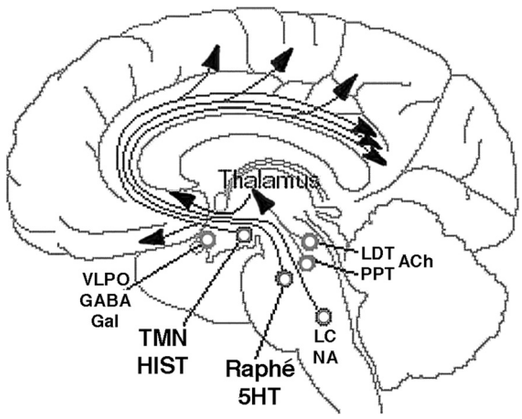

Figure 1. The ascending arousal system sends projections from the brainstem and posterior hypothalamus throughout the forebrain. Neurons of the laterodorsal tegmental nuclei and pedunculopontine tegmental nuclei (LDT and PDT) send cholinergic fibers (ACh) to many forebrain targets, including the thalamus, which then regulate cortical activity. Aminergic nuclei [remaining circles] diffusely project throughout most of the forebrain, regulating the activity of cortical and hypothalamic targets directly. Neurons of the tuberomammillary nucleus (TMN) contain histamine (HIST), neurons of the raphe nuclei contain 5-HT and neurons of the locus coeruleus (LC) contain noradrenaline (NA). Sleep-promoting neurons of the ventrolateral preoptic nucleus (VLPO) contain GABA and galanin (Gal).

Histamine blood levels have been shown to oscillate in AD patients. Levels correlate positively with delta activity (a measure of slow wave sleep) in central, temporal and parietal areas in the right hemisphere. Increasing histamine levels in AD progression seem to be of peripheral origin but active within the CNS.[9] It appears that a very subtle dysfunction in brain histamine might contribute as a secondary event to the aetiopathogenesis of AD related sleep disorders.[1] Therefore, pharmacological targeting of central histaminergic receptors may prove useful in the treatment of AD related sleep disorders.

In an attempt to compensate for lost SWS and REM overnight sleep, individuals with AD

frequently nap during the day. However, because AD naps rarely reach REM stage, they poorly atone for lost hours.[1] In fact, an increase in nap frequency and duration is often used to diagnose the progression of mild/moderate AD to severe AD. Prominent hypersomnolence or severe insomnia is typically only found in later stages of the disease.[2] A recent study suggests that daytime napping might be a risk factor for mortality, perhaps because it is a marker for significant nocturnal sleep disturbance or because blood pressure declines as it does during sleep at night. This increases the risk of cardiovascular or cerebrovascular accidents.[5] Severe AD patients may spend as much as 40% of their time awake in bed often in a confused state, thus increasing the need for daytime sleep.[10]

AD sleep disorders may also be manifestations of psychological issues in a patient who suffers from the disease. For example, AD in its progressive forms may cause agitation, depression, anxiety, anger, hallucinations and delusions.[10] Because depression and mania cause insomnia in non-demented patients, AD sleep disorders may be easily treatable by targeting these behavioral mechanisms. Currently, anti-depressants are widely prescribed in treatment of AD sleep related disorders, a remedy which leads many physicians to believe erroneously that behavioral pathologies are the only causes of AD related sleep disorders.

Current treatments for improving sleep in AD fall into three broad categories: pharmacological, cognitive-behavioral or psycho-educational strategies and biological/circadian therapies.[10] This paper will concentrate on pharmacological and biological/circadian therapies. Behavioral approaches will be dealt with from a pharmacological viewpoint, but this should not diminish the importance of cognitive therapy. Biological/circadian therapies are currently the treatment of choice as pharmacological therapies that specifically target AD sleep disorders are still in their infancy. Sleep disturbance is sometimes a side effect of the cholinomimetic drugs as cholinergic neurons in the brainstem are particularly active in the wakeful state. Evidence of sleep disturbance as a reaction to these drugs is well documented in the young but results are less conclusive in the elderly.[3] Apart from anti-depressants, benzodiazepines and other hypnotics are widely prescribed as pharmacological intervention. These have been clinically shown to be habit forming and are less than ideal. Thus, the treatment benefits of sedating medications in persons with a progressive dementing illness may not outweigh the potential risks of their continued use for sleep and night-time agitation.

Treatment of AD sleep related disorders is not just necessary for the improvement of patient lifestyle. It has been demonstrated that there is a significant correlation between quality of diurnal sleep and better cognitive performance.[3] Effective treatment may theoretically delay or even reverse the progression of dementia. As a greater understanding of the pathophysiological processes in AD are elucidated, pharmacological intervention is emerging as a hopeful treatment strategy in treating a critical component of AD progression.

DRUGS AFFECTING SLEEP/WAKE CYCLE IN ALZHEIMER'S DISEASE

Pharmacological interventions involve tricyclic anti-depressants, benzodiazapines, non-benzodiazepines, classical antipsychotics, atypical antipsychotics and antihistamines (table 1).

Table 1. Drugs affecting the sleep/wake cycle in Alzheimer's Disease.[3]

Drug	Recommended Dose (mg/day)	Potential Side Effects
Tricyclic Antidepressants		
Nortriptyline	10-75	Anticholinergic Effects
Trazodone	25-75	Orthostatic Hypotension
Benzodiazepines		
Lorazepam	0.5-2	Lethargy,Confusion,ataxia
Oxazepam	10-30	Dependence
Triazolam	0.0625-0.125	
Non-Benzodiazepines		
Zolpidem	5-10	
Zaleplon	5-10	
Classical Antipsychotics		
Chlorpromazine	10-100	Sedation,anticholinergic
Haloperidol	0.5-1	Extrapyramidal symptoms
Thioridazine	10-100	Sedation, anticholinergic
Atypical Antipsychotics		
Clozapine	25-100	Sedation, agranulocytosis
Risperidone	1-6	Orthostatic hypotension

These classes of medications target a wide spectrum of sleep disorders and therefore fail to target AD night-time agitation *per se*. Furthermore, clinical case studies of these drugs that pertain specifically to AD are sparse.

Benzodiazepines

Seven diagnosed AD males were placed on the standard geriatric dose (0.125mg) of the short acting benzodiazepine, triazolam. Exclusion criteria for the experiment were previous sleep disorders and/or use of a drug with prominent cholinergic or anti-cholinergic effects. Because the leading AD medications are cholinomemetic drugs, the study did not monitor drug-drug interactions between the two classes of drugs in AD patients. The patients were monitored for alteration in sleep activity and memory function. Group data failed to reveal any significant effect of triazolam on total sleep time at night, latency to sleep onset, number of nocturnal awakenings,

total sleep time during the day or mean level of activity during night or day in the six subjects with complete actigraph data.[11] Furthermore, typical benzodiazepine side effects such as psychomotor and memory impairment, rebound insomnia and dependence were reported. This study was significant in that it was the first study to monitor the effects of a sleep hypnotic in AD that had been proven effective in normal geriatric patients. The researchers concluded that the disrupted sleep of typical AD patients might not be comparable to conditions that cause insomnia and/or chronic sleep deprivation in normal patients. Benzodiazepines also have minimal effects on sundowning i.e. a deterioration of AD symptoms after sunset.[3] Thus, the side effects of prescribed benzodiazepine sleep agents may in fact prove to be detrimental to AD patients rather than therapeutic.

Cholinomimetic Drugs

Acetylcholine release within the basal forebrain changes significantly as a function of sleep and wakefulness.[9] Anticholinesterases are the drugs of choice in slowing the degenerative symptoms in AD. Their effects on sleep are variable but more research is needed to examine the effects of these drugs on sleep in AD patients.[10] No extensive research has been conducted on the anticholinesterase Tacrine in relation to sleep and AD. Rivastigmine, an acetylcholinesterase inhibitor, has been shown to reduce REM sleep in younger adults but in older adults had no negative affect on REM duration, sleep efficiency, sleep latency, number of awakenings or time awake.[3] Another anticholinesterase inhibitor, Donepezil, was shown to improve cognitive performance in AD patients but over 40% of the patients had insomnia as a side effect.[12]

As was seen in the triazolam study, cholinomimetic drugs are often excluded due to possible drug-drug interactions.[3] This, coupled with the sparse knowledge underlying AD sleep-related disorders, means that the effects of anticholinesterases and pharmacological hypnotic agents are still largely unknown. The fact that many AD pathological symptoms overlap those associated with sleep deprivation further complicates treatment as differentiation between the two is largely speculative. Future pharmacological agents that symptomatically target AD sleep related disorders must harmonise with those pharmacological agents that target the disease *per se*.

Histaminergic Drugs

Histamine is involved in the control of vigilance, sleep and wakefulness, as well as in the modulation of circadian rhythmicity. This H_1-mediated arousal response has also been demonstrated by EEG studies. Blood histamine levels increase as AD progresses but the extent to which this affects the CNS is a topic of hot debate. Laboratory experiments by Novoa *et al* indicate that the histaminergic system seems to be involved in pathological states relating to the neurodegeneration as seen in AD. Histamine levels increase as AD progresses; concurrently, the severity of sleep disorders in AD also increases as the disease progresses (Figure 2).[9]

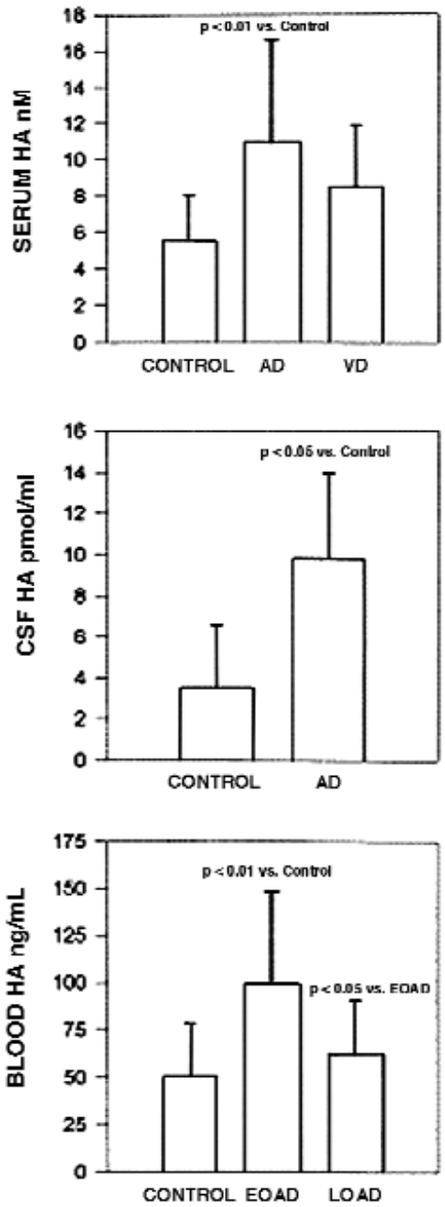

Figure 2. Histamine (HA) levels in serum of Alzheimer's disease (AD) and vascular dementia (VD) patients with respect to control subjects; histamine levels (HA) in cerebrospinal fluid (CSF) of Alzheimer's disease (AD) compared to control subjects; whole blood histamine levels in early-onset (EOAD) and late-onset (LOAD) AD patients with respect to control subjects. Results are expressed as mean +/- SD.

The fact that first generation H_1 receptor anti-histamines induce drowsiness further supports the argument that histamine plays an integral role in arousal. Considering that the histaminergic system acts as a regulatory center for brain activity, its malfunctioning can alter important pathways implicated in motivated behaviours, behavioural disorders, control of waking state, neuroendocrine and cardiovascular regulation.[9] When the role of histamine in arousal is coupled with our relatively good understanding of histaminergic receptors, a key pharmacological target arises that could help provide effective AD sleep related treatment.

Melatonin

Melatonin is a peptide hormone produced by the pineal gland that influences sleep-wake cycles and other circadian rhythms. It has a sedative effect and is currently used to treat sleep disorders and jet lag.[13] The most extensive research regarding AD sleep related disorders have focused on the circadian affect of exogenous melatonin in the neurodegenerative model. Across all studies researchers concluded that melatonin secretion is altered in AD, but post-mortem examination failed to show the classic pathological finding of ß/A_4 deposition in pinealocytes.[12] However, a deficiency of CSF melatonin is postulated to be critical for the development of AD.[14,15] AD patients simultaneously showed significantly reduced amplitude, larger variation of peak times and diminished amount of total secretion in the melatonin secretion rhythm compared with their non-demented counterparts (figure 3).[15] Hypotheses for this include:

1- Organic deterioration of the circadian time-keeping system including the suprachiasmatic nucleus and its afferent and efferent projections.[8]

2- Decreased social interaction, encounter with the sun and general external sensory stimulus due to environmental factors commonly found with individuals with AD.[12]

3- Studies have found that melatonin levels are increased in AD patients during daytime and that these patients do not react equally to bright light as their non-demented counterparts.[13]

Preliminary reports suggest that melatonin decreases sundowning in AD patients. Furthermore, in vitro experiments found that melatonin functions as an anti-oxidant and neuroprotector in rat and primate brain tissue.[13] Inadequate melatonin in AD allows hydroxyl radicals produced by mitochondrial complex IV to damage mitochondria and initiate a cascade of oxygen radicals that causes the neuropathological changes in AD.[12] Thus, in addition to its neuroprotective qualities, melatonin has been shown to help correct the aberrant retina-SCN-pineal axis required for normal sleep in AD patients.[12] Irrespective of the method of assessment, melatonin showed positive effects in insomniac patients in most studies.[16] Although it is imperfect, melatonin is currently accepted to be the most effective pharmacological therapy for correcting AD sleep related disorders.

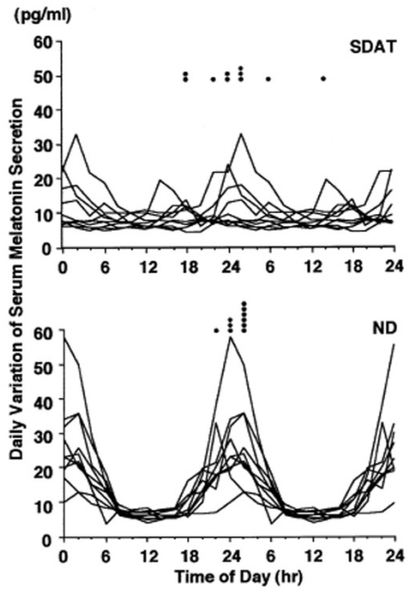

Figure 3. Raw data plots of daily variation of serum melatonin concentrations in the SDAT and ND groups. The ND group formed regular circadian patterns of melatonin secretion with nocturnal increase and sufficient daytime suppression. In contrast, the SDAT group showed irregular patterns of melatonin secretion with reduced peak secretion levels and large variations in peak secretion time indicated as small black points. Three patients in the SDAT group showed peak secretion times during the daytime, whereas none in the ND group did.[15]

BRIGHT LIGHT THERAPY (BLT)

Natural sunlight regulates the body's natural daily arousal system, the circadian rhythm, via a direct neural pathway from the retina to the pineal gland. Light suppresses the release of melatonin which can stimulate ML_{1A} and ML_{1B} receptors. Because AD patients have altered circadian activity, artificial stimulation of their pineal gland may effectively normalise their circadian rhythm. In a study conducted by Nippon Medical School in Tokyo, AD patents were treated with bright light therapy (3000 lux; 1 lux equals a light intensity equivalent to 1 lumen/m^2) daily from 9-11am. Treatment did not slow the cognitive degeneration as defined by the Clinical

Dementia Rating in severe AD patients. However, it did significantly decrease the percentage of nap time during the day and increase the percentage of night-time sleep in all 27 patients.[17] The therapeutic benefit was more pronounced in mildly demented patients and less marked in severely demented patients. Furthermore, in the treatment of AD with BLT, it was shown that short wavelength light is more effective at suppressing melatonin than long wavelength light.[13] These findings are clinically relevant for two main reasons:

1- BLT is a safe, non-pharmacological alternative to drugs that react poorly with patients with AD sleep related disorders.

2- The adaptations observed in circadian neuronal systems of aged individuals evidence neuronal plasticity even in severely demented patients.

Because community dwelling AD patients are exposed to light greater than 1000-lux less than 40 minutes per day on average, BLT may provide a safe, affordable solution to many AD related circadian abnormalities.

Vitamin B_{12}/Cobalamin

The effects of vitamin B_{12} and BLT on 28 AD patients were monitored to determine their effects on the sleep-wake cycle.[18] It is theorised that the methylation of homocysteine to methionine decreases levels of free homocysteine in the CNS, thereby reducing the neurotoxic effect of elevated homocystine levels on melatonin suppressing neurons. Thus, increased levels of vitamin should further suppress melatonin during BLT and thus have a positive psychotropic alerting affect.[18] Patients with early-stage AD showed improved vigilance and decreased duration of daytime naps while receiving vitamin B_{12} and BLT in comparison to BLT alone.[18] Those patients who received vitamin B_{12} exclusively failed to show an improvement. No difference was detected in severely demented individuals. Thus, the study concluded that vitamin B_{12} increased the sensitivity of BLT rather than having a direct pharmacological effect.

CONCLUSION

The pathogenic processes underlying AD severely impair quality and quantity of sleep as the disease progresses. Currently, this aspect of AD remains largely untreated as the exact pathology in AD sleep disorders continues to elude researchers. A myriad of neurotransmitters and hormones contribute to the regulation of the sleep-wake cycle. These have been shown to be present in increasingly defective proportions as AD becomes more severe. The use of pharmacological agents in treatment carries some risk as side effects may outweigh therapeutic benefit. Consequently, biological and circadian therapy is currently the most effective treatment in AD sleep disorders. Because the pathogenicity in AD sleep disorders mirrors much of pathogenicity in AD memory disorders, elucidation of the neurophysiological processes in AD sleep disorders may provide valuable information in treatment of memory loss in AD.

AD related sleep disorders are an integral and pervasive aspect of the disease and can cause widespread perceptual and emotional disturbances. Consequently, immense strain is placed on family members, caregivers and the patient. Both pharmacological and non-pharmocological agents have been used to confront this aspect of the disease yet neither has proved an adequate solution. As more of the pathophysiological processes are revealed, pharmacological intervention is emerging as a hopeful therapeutic strategy in treating this critical component of AD.

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An Electrogastrographic Study of Gastric Myoelectrical Activity in Acute Pancreatitis

Róisín English
4th year Medicine

ABSTRACT
Objectives: Oral feeding is traditionally prohibited in acute pancreatitis patients. This is partly due to an assumption that acute pancreatitis is associated with some degree of gastrointestinal atony, and that oral feeding would thus not be tolerated. Gastric motility has never been studied in acute pancreatitis. The aim of this study was to determine whether gastric myoelectrical activity as measured by electrogastrography (EGG) was altered in a group of acute pancreatitis patients compared to a group of healthy controls. **Methods:** Nine acute pancreatitis patients and thirteen healthy volunteers were assessed. Three electrodes were used to record EGG for 30 minutes before the subject drank 500 ml of water, and then for 60 minutes afterwards. EGG parameters measured included dominant frequency, power ratio and percent normogastria. **Results:** In the control group, the dominant frequency and percent normogastria were found to decrease significantly after water ingestion, as expected. The mean power ratio in the control group was greater than 1, showing an increase in power after ingestion of water. The acute pancreatitis patient group (n = 9) studied on the day following admission showed no significant difference in any of the EGG parameters measured when compared to the control group. **Conclusion:** The results of this study show that there is no abnormality in gastric myoelectrical activity in acute pancreatitis. This indicates, due to the close relationship between myoelectrical activity and motility, that gastric motility is normal in acute pancreatitis.

INTRODUCTION

Acute pancreatitis is an acute inflammatory process of the pancreas, a mixed gland that secretes both digestive enzymes and endocrine hormones such as insulin and glucagon. The effects of acute pancreatitis on gastric motility have never been studied. It has been shown that gastric motility is regulated by gastric myoelectrical activity, which can be measured cutaneously by EGG.[1]

Gastric myoelectrical activity consists of slow waves of depolarisation originating from a pacemaker area along the greater curvature of the stomach and propagating with increasing velocity towards the pylorus at a rate of approximately 3 cycles per minute (cpm).[2] These slow waves are always present and determine the propagation and maximum frequency of contractions of the gastric smooth muscle. Electrogastrography measures gastric myoelectrical activity by cutaneous electrodes placed on the abdominal skin over the stomach, recording a sinusoidal wave reflecting the overall electrical activity of the stomach.[3] Gastric myoelectrical activity, as measured by EGG, has been shown to modulate gastric motility.[4] Abnormalities in myoelectrical activity are associated with motility disturbances, such as those seen in gastroparesis, motion sickness, post-gastric surgery and other clinical disorders.[5]

The parameters most commonly measured by an EGG are dominant frequency, percent normogastria and power ratio. The dominant frequency of this EGG wave has been shown to be the same as that of the gastric slow wave measured from both serosal and mucosal internal electrodes.[6] Frequencies between 2 and 4 cpm are considered normal. Frequencies above and below this range are considered tachygastria and bradygastria respectively.[5] The dominant frequency and percent normogastria are indicators of normal gastric function and the rhythmic stability of the motor pattern.[4] It is generally agreed that the absolute value of EGG amplitude is not useful since it could be related to the variable distance between the stomach and the recording electrodes.[7] Therefore, a ratio of the pre: post- stimulus power is used to cancel out these variables. The stimulus used in clinical practice is usually a standardised meal. The dominant power ratio may be reliably used as a measure of spike activity and contractile strength of the stomach wall.[6] In clinical practice, there are three criteria used to determine whether an electrogastrogram is normal. The dominant frequency must be within the range of 2-4 cpm, the percent normogastria post-stimulus must be greater than 70% and the power ratio must be greater than 1.[5]

The non-invasive nature of the EGG procedure means it can feasibly be used in this critically ill group of acute pancreatitis patients. The stimulus in this case had to be water, because oral feeding of acute pancreatitis patients is traditionally prohibited. This prohibition is based on two assumptions. The first is that feeding the gut will stimulate pancreatic exocrine secretion, thus aggravating the clinical condition. There have been studies contradicting this view, showing that while oral feeding may indeed induce exocrine

secretions, it does not exacerbate the disease.[8,9] Secondly, there exists a belief that acute pancreatitis is associated with gastric and intestinal atony, and that oral feeding would thus not be tolerated.[10] In this study, it is proposed to investigate the assumption that there is a gastric motility abnormality associated with acute pancreatitis. Electrogastrography will be used in order to determine whether gastric myoelectrical activity is altered in a group of acute pancreatitis patients compared to a group of healthy controls.

METHODS

Nine acute pancreatitis patients (3 men, 6 women) were assessed on the day following admission to St. James's Hospital, Dublin. The average age was 51.44 ± 6.38, ranging from 23 to 90. Diagnosis of acute pancreatitis in all patients was based on a suggestive clinical picture and consistent morphological findings on computed tomography (CT) scan. Clinical and biochemical data from these patients are seen in table 1. Only intravenous electrolyte solutions, antibiotics and analgesics were administered before the study. No patients developed any major complications of acute pancreatitis and there were no mortalities. Thirteen healthy volunteers (6 men, 7 women) with no gastrointestinal symptoms were assessed on one occasion only. The average age in this control group was 41.46 ± 4.88, ranging from 22 to 85. The St. James's Hospital Ethics Committee approved the study protocol and written consent was obtained from each subject prior to the study.

EGG Measurement

Gastric myoelectrical activity was measured non-invasively by EGG. Each subject was fasting for at least six hours (usually overnight) prior to the investigation. The skin over the abdomen at the recording sites was shaved, if necessary, to remove excess hair and carefully abraded with sandy skin-prep jelly to reduce the impedance between electrodes. Three silver/silver-chloride electrodes were placed on the skin over the stomach after application of electrode gel, as can be seen in figure 1. The first recording electrode was placed halfway between the xiphoid process and the umbilicus in the midline. The second recording electrode was placed 45^{o} to the left and 5 cm above the first. The reference electrode was placed on the left flank underneath the ribcage. After placement, the resistance between the electrodes was checked using an ohmmeter and found to be below 5kΩ in each subject. The electrodes were connected to a portable EGG recording device (Digitrapper EGG, Synectics Medical Inc, Irving, Texas). Subjects were lying in a semi-recumbent position and were asked not to move during the recording. Data was recorded for 30 minutes before the subject drank approximately 500ml of water, and then for 60 minutes afterwards. The EGG data stored on the recorder were downloaded to an IBM personal computer and underwent spectral analysis at 2Hz. A Fast Fourier Transform (FFT) was applied to consecutive 256 second signal stretches that have an overlap of approximately 75%. This resulted in a series of minute-by-minute frequency spectra. The dominant frequency of the EGG was measured in contractions per minute and the dominant power, defined as the amplitude of the EGG at the dominant frequency, was measured in dB. The power ratio, which is defined as the ratio of the amplitude of the dominant frequency pre-water and post-water, was also assessed. Other parameters recorded were percent bradygastria (percent time that the dominant frequency lay in the range 0-2cpm), percent normogastria (percent time that the dominant frequency lay in the range 2-4cpm) and percent tachygastria (percent time that the dominant frequency lay in the range 4-10cpm).

Blood samples were taken from each acute pancreatitis patient on the day of admission. Serum amylase and Glasgow score were recorded as shown in table 1. The sex, age and cause of acute pancreatitis were also recorded for each patient. All data were expressed as mean ±

Table 1. Clinical characteristics and biochemical data of patients with acute pancreatitis.

Case No.	Age	Sex	Aetiology	Amylase (IU/L)	Glasgow Score
P1	23	F	Gallstone	316	0
P2	52	F	Gallstone	652	2
P3	90	F	Gallstone	1076	3
P4	37	F	Alcohol	382	1
P5	59	M	Alcohol	779	3
P6	37	F	Alcohol	35	2
P7	59	F	Gallstone	530	4
P8	60	M	Alcohol	89	5
P9	46	M	Alcohol	1447	1

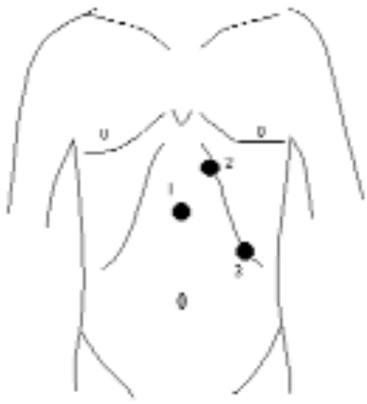

Figure 1. Placement of EGG electrodes is as shown. The electrodes are then connected to a portable EGG device and data recorded for 30 minutes before and 60 minutes after ingestion of approximately 500 ml of water.

standard error and $p<0.05$ was considered statistically significant. Because the data were not normally distributed due to the small sample size, non-parametric statistical tests were used to determine significance. Wilcoxon's signed rank sum test was used to compare EGG parameters within groups and Wilcoxon's unpaired test was used to compare between groups.

RESULTS

In the control group of 13 subjects, the dominant frequency was found to decrease after water ingestion from 2.97 ± 0.087 cpm to 2.58 ± 0.12 cpm ($p = 0.05$, $n = 13$), as seen in figure 2. The frequency of the slow waves remained within the normal 2 to 4 cpm range for over 80% of the recording time, and this percentage decreased significantly after water (88.56 ± 3.074 versus 82.31 ± 2.393 cpm, $p = 0.0403$, $n = 13$). There was a significant increase in the percent bradygastria after water (9.35 ± 2.684 versus 12.94 ± 2.447 cpm, $p = 0.0421$, $n = 13$). The mean power ratio in the control group (figure 3) was greater than 1,

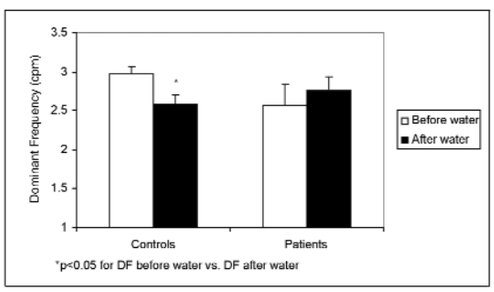

Figure 2. Changes in dominant frequency (cpm) with ingestion of water in control and patient group. There is a significant decrease in the mean dominant frequency after water in the control group, (n=13) but no significant change in the patient group (n=9). Data are expressed as means ± SE.

showing an increase in power after ingestion of water (mean power ratio=4.18±1.482).

The acute pancreatitis patient group (n = 9) studied on the day following admission showed no significant difference in any of the EGG parameters measured when compared to the control group. The dominant frequency before water was 2.58 ± 0.263 cpm in the acute pancreatitis group and 2.97 ± 0.087 cpm in the control group ($p = 0.13$). Post-water dominant frequency also failed to reach significance between these groups (2.76 ± 0.178 cpm for patients versus 2.58 ± 0.12 cpm for controls, $p = 0.17$). The mean dominant frequency in the patient group showed an increase after water (as opposed to the decrease seen in the control group), but this increase was not significant (2.58 ± 0.263 versus 2.76 ± 0.178 cpm, $n = 9$, $p = 0.29$).

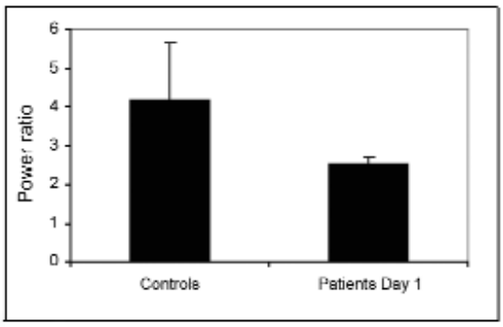

Figure 3. Power ratios (PR) in each group. The power ratio is the ratio of the amplitude of the dominant frequency pre: post water. Both groups showed an increase in power after water ingestion. There was no significant difference in PR between controls and patients.

No significant differences were observed when comparing the pre- and post- water percent bradygastria, normogastria and tachygastria between the patients on day of admission and the control group. The mean percentage of slow waves with a frequency between 2 to 4 cpm (normogastria) was 81.31 ± 4.668% before water and 85.64 ± 5.059% after water. The mean power ratio in the patients group was 2.53 ± 0.584, which shows that the amplitude of the EGG signal increased after water, as it did in the control group. There was no significant difference between power ratios in the Patients Day 1 group when compared to the control group (figure 3).

DISCUSSION

The aim of this study was to determine whether or not gastric myoelectrical activity was altered in acute pancreatitis patients compared to a group of normal controls. Electrogastrography was used to measure gastric myoelectrical activity, due to its non-invasive nature and proven accuracy in recording the gastric slow wave.[6]

Controls

EGG parameters obtained from the control group remained within the normal ranges used in clinical practice.[5] As expected, ingestion of a water stimulus caused a significant change in the dominant frequency as measured by electrogastrography (see figure 2). The decrease in dominant frequency seen in this group of normal healthy subjects is in agreement with results obtained in other studies. A study by Chen & McCallum investigated the effects of water ingestion on the postprandial electrogastrogram.[11] During the first ten minutes after drinking water, the dominant frequency was lower than or equal to the preprandial value in nine out of ten subjects. In a separate study of ten subjects, a decrease in the dominant frequency was found in all ten subjects after water ingestion.[6] In the present study, water also induced a significant reduction in the percent time that the frequency remained within the normal 2 to 4 cpm range (normogastria). Since the two groups mentioned previously did not measure percent normogastria, this reduction was not previously documented. The mean power ratio in the control group was greater than 1, as expected (figure 3), and therefore indicated increased contractility of the stomach following ingestion of water.

Acute Pancreatitis Patients

No significant differences were observed in any of the EGG parameters measured for the patient group assessed on day after admission when compared to the control group. In fact, water did not induce any significant changes in EGG parameters in the patient group assessed on the day of admission. The reason for this may be the smaller sample size in the Patient Day 1 group (n = 9) compared to the control group (n = 13). However, all parameters measured in acute pancreatitis patients were within the normal ranges set out by Lawlor *et al*.[5] In this study, no abnormality in gastric myoelectrical activity assessed by EGG was observed in acute pancreatitis patients.

Oral Feeding in Acute Pancreatitis

Oral feeding of acute pancreatitis patients is traditionally prohibited. This prohibition is based on two assumptions. The first is that feeding the gut will result in stimulation of enteral hormone secretion and direct activation of pancreatic enzymes, thus aggravating the severity of the inflammation and worsening the pain. A study on orally fed acute pancreatitic rats questions this convention.[8] Acute pancreatitis was induced in all rats by ligation of the main biliopancreatic duct, and rats were then fed either orally or parenterally. While rats fed orally did indeed have higher levels of enteral enzymes such as amylase, histopathological examination of the pancreas revealed more severe inflammatory changes in those rats fed parenterally. The authors suggest that some enteral hormones, such as motilin and cholecystokinin, have a protective effect, thus limiting the inflammation process in the pancreas. Additionally, intestinal stasis due to lack of oral feeding may promote bacterial translocation and further aggravate local and systemic complications of acute pancreatitis.[8]

Further evidence to suggest that oral feeding does not exacerbate acute pancreatitis comes from a clinical trial involving 34 acute pancreatitis patients who were randomised into enteral and parenteral groups.[9] Patients with mild to moderate disease were fed orally on a clear fluid diet with nutrition supplements and those in the parenteral group via a peripheral intravenous line. Patients with severe disease were fed enterally via a nasojejunal feeding tube or parenterally via a central venous catheter. Clinical outcome parameters such as systemic inflammatory response syndrome, sepsis, multiple organ failure and hospital stay were all improved in the enterally fed patients. C-reactive protein and Modified Glasgow score were also significantly reduced in patients receiving oral or nasojejunal feeding. [9]

Gastric Motility in Acute Pancreatitis

The assumption that some degree of gastrointestinal atony exists in acute pancreatitis patients is the second reason for the avoidance of oral feeding in this patient group. In this study, it has been found that gastric myoelectrical activity of acute pancreatitis patients is normal on the day after admission to hospital. No other studies investigating gastric motility in acute pancreatitis were found in the literature. There have been several studies that show that gastric motility is altered in chronic pancreatitis. Accelerated gastric emptying of a low-energy liquid meal and a shorter duration of the interdigestive motor cycle were observed in patients with severe chronic pancreatic insufficiency. Pancreatic enzyme supplementation was associated with a longer fed pattern and decelerated gastric emptying.[12] The fact that enzyme supplementation, and therefore decreased malabsorption, reversed the changes observed in pancreatic insufficiency suggests that the motility changes are caused by malabsorption and increased nutrient delivery to the small intestine. Another study reiterated these findings and further showed that the motility changes observed in chronic pancreatitis were correlated with the degree of exocrine pancreatic insufficiency.[13] In chronic pancreatitis patients, the fed motor pattern was

longer, antral motility indexes were reduced and the interdigestive cycle length was shorter than in controls. These abnormalities were much more pronounced in chronic pancreatitis patients with exocrine pancreatic insufficiency compared to chronic pancreatitis patients without exocrine pancreatic insufficiency, and pancreatic enzyme supplementation reversed the changes towards normal.[13] Therefore, gastric motility is altered in chronic pancreatitis due to exocrine pancreatic insufficiency.

In acute pancreatitis, levels of exocrine pancreatic enzymes measured by a duodenal intubation perfusion technique remain within the normal range. Secretion of pancreatic amylase, trypsin and chymotrypsin continued to be cyclical as normal in acute pancreatitis patients with a serum amylase threefold the upper limit of normal and clinical evidence of the disease. No statistical differences were found in pancreatic enzyme output per hour within a cycle or per secretory peak, as compared to a group of healthy controls.[14] Even though exocrine pancreatic enzyme levels are normal, there is a possibility that other pancreatic hormones and peptides could be involved in the modulation of gastric motility in acute pancreatitis. Amylin, for example, is a peptide synthesised in the endocrine beta cells of the pancreas and has been shown to inhibit gastric emptying in rats.[15] Pancreatic polypeptide stimulates gastric motility under normal conditions by acting indirectly via a vagal cholinergic mechanism.[1] It is interesting that hyperglycaemia has also been shown to inhibit gastric motility and this effect is mediated by impaired vagal activity.[16] Although pancreatic polypeptide concentrations have been found to be normal in acute pancreatitis, the concentrations of amylin have yet to be studied.[14]

The results of this study show that there is no abnormality in gastric myoelectrical activity in acute pancreatitis. This indicates, due to the close relationship between myoelectrical activity and motility, that gastric motility is normal in acute pancreatitis. Further studies are needed in order to fully investigate gastric motility in acute pancreatitis. These would involve the use of more invasive motility tests, such as gastric emptying of an isotope-labelled meal and antroduodenal manometry, which directly measure motility rather than myoelectrical activity. If motility were established to be normal using these techniques, then a review of the nutritional management of acute pancreatitis patients would surely be necessary and the prohibition on oral feeding could feasibly be lifted.

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Subcloning and Transformation of a Portion of the Gene Encoding the ISG-75 N-terminal Domain of *Trypanosoma brucei*

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ABSTRACT:

Objectives: African trypanosomiasis is a parasitic disease caused by *Trypanosoma brucei* and transmitted by the tsetse fly. Trypanosomes evade the immune system by altering their surface structure, which contains a layer of 10^7 variant surface glycoprotein (VSG) that partially shield a number of underlying invariant surface glycoproteins (ISGs).[1,2] Understanding invariant surface glycoproteins has been the recent focus of study for vaccine development and chemotherapy. Since its discovery, ISG-75 has received little study despite the fact its sequence is unique and not related to any other ISG. The focus of this project was to subclone the portion of the gene encoding the 440 amino acid N-terminal domain of ISG-75. The recombinant domain was purified and used to raise antibodies for use as a reagent to determine the orientation of ISG-75 in live bloodborne trypanosomes. The orientation of this protein is important, both for academic reasons in the attempt to understand its function and practically, as a possible target for parasite control by chemotherapy or immune control via a single chain. This involves the production of a single domain recombinant antibody that would be small enough to pass the VSG barrier and reach its ISG target. Understanding which part of the protein is exposed externally on the cellular surface is critical in selecting the portion of the protein to target. **Methods:** Polymerase Chain Reaction (PCR) was performed using genomic DNA to obtain the N-terminal domain of ISG-75. The gene fragment encoding the N-terminal domain of ISG-75 and the pET-21a vector were successfully double digested with the restriction enzymes, Nde1 and Not1. The insert and vector were cut from 1% agarose gels and extracted from the gel material. They were then ligated together and transformed into *E. coli*. **Results:** The N-terminal domain measured 1354 basepairs after PCR. After incubating the insert and vector with the restriction enzymes the N-terminal domain was visible in the appropriate region. Two smaller band fragments 600 and 754 basepairs in length were also identified. The pET-21a vector was found in the appropriate region and measured 5443 basepairs in length. *E. coli* colony counts where performed on both the ligation mixture and control LB agar plates. Thirteen colonies were found on the ligation mixture plate.

INTRODUCTION

Trypanosoma brucei, a parasitic protozoan hemoflagellate, is the causative agent of sleeping sickness in humans and nagana disease in cattle.[3] This parasite has medical, veterinary, and economic significance.[4] Humans are infected with *T. brucei* during a blood meal by the tsetse fly. The parasite migrates through the bloodstream and into the lymphatic system, multiplying by binary fission.[5] The gene encoding the variant surface glycoprotein (VSG) is switched every eight to ten days from amongst some 1000 existing gene variants. This allows the parasite to evade the humoral immune response. Due to the large number of VSG genes and their rapid rate of switching the VSG is thought to be unsuitable as a vaccine target.[6-8]

This has shifted the focus of study towards the invariant surface glycoproteins (ISGs) in the hope that their invariant nature will make them better vaccine candidates than a constantly changing VSG. Gene cloning and sequencing experiments indicate that most ISGs are composed of a large extracellular domain, a single transmembrane α-helix, and a small intracellular domain. They are also uniformly distributed across the cell surface with an abundance of 0.5% of that of the VSG. ISGs were first identified using non-penetrating surface-labeling techniques. First, surface biotinylation was used in the discovery of ISG-60, ISG-65 and ISG-75.[9,10] ISG-64, ISG-70 and ISG-100 were found using enzyme catalyzed surface radioiodination techniques.[11,12] Several other surface proteins have also been characterized in detail including the heterodimeric transferrin receptor,[13-15] a Ca^{2+}-regulated adenylate cyclase,[16,17] and the glucose transporter.[18] These proteins represent additional vaccine candidates because they do not undergo antigenic variation and deliberate immunization against such antigens may provide protection against the disease.

ISG-75 is unique and unrelated to any other ISG. The genes encoding ISG-75 are present at two loci, A and B, in tandem arrays containing five and two copies, respectively. In the A locus, a single gene encodes a structural isoform of ISG-75. At least one copy of the gene

(SW478) encoding this unique isoform of ISG-75 is present in every strain tested. ISG-75 contains a large 440 amino acid N-terminal folding domain, a short hydrophobic trans-membrane domain, and a short 34 amino acid C-terminal folding domain. It has been postulated that the N-terminal domain is external and C-terminal domain is internal to the plasma membrane.[19] However, there is little experimental evidence to validate this orientation claim for ISG-75. In ISG-75, the N-terminal domain consists of 16 tyrosines and the C-terminal contains no tyrosine. Both domains contain lysine. Given that radioiodination only detects tyrosines, this would account for the failure of this method to detect the protein in intact cells. Biotinylation modifies lysines, and detects lysine regardless of the orientation. Therefore, because tyrosine was not detected using radioiodination and tyrosine is only on the N-terminal domain, it is likely that the N-terminal is internal and the C-terminal external to the plasma membrane.

MATERIALS AND METHODS

Preparation of the N-terminal domain

Oligonucleotide PCR Primers:

The genomic DNA encoding ISG-75 was amplified by PCR with forward and reverse primers. The forward primer,
5'-ggaattccatatggaggagctctctgttgcg-3',
and the reverse primer,
5'-actagagtggcggccgctcacttcgttgtcccaactca-3',
were synthesized by the MWG-Biotechnology Oligonucleotide Laboratory.

PCR:

The specific primers were used to perform PCR thermal cycles (1 denaturing cycle of 94°C for 1 minute, 35 annealing cycles of 94°C for 30 seconds, 62°C for 1 min 20 s, 72°C for 3 min and 1 polishing cycle of 72°C for 6 min). The reaction was performed in a 50µl reaction volume containing: 10mM Tris-HCL, pH 8.3, 50mM KCl, 1.5mM $MgCl_2$, 0.2mM dNTPs, 50ng DNA template, 0.2 µmol L^{-1} of each primer, 2.5U Taq DNA polymerase (Sigma). The amplification was performed in a thermal cycler (PCR Sprint).

Restriction Digest:

The amplified partial gene product encoding the N-terminal domain of ISG-75 was double digested with the restriction enzymes Nde1 and Not1 at 37°C overnight. The digested product was electrophoresed on a 1% agarose gel using Tris-acetate-EDTA (TAE) buffer. The gel was visualized using ethidium bromide and a long wavelength ultraviolet light source. The desired band, 1354 basepairs, was cut from the gel and extracted using the Qaiger QIAquick gel extraction kit protocol.

Preparation of the vector

The expression vector, pET-21a, was used. The multicloning site contains Nde1 and Not1 restriction sites. This vector also contains an ampicillin resistant gene, which facilitates growth on ampicillin Luria-Bertani (LB) agar plates. A sample of the purified vector was digested with Nde1 and Not1. The digested product was electrophoresed on a 1% agarose gel, the band containing the cut vector excised, and extracted from the gel using the methodology stated above.

Quantification of the products

The concentrations of both the N-terminal domain and pET-21a vector were determined by comparison with a standard (Invitrogen Ready-Load 1Kb DNA ladder).

Ligation

A ligation of the N-terminal domain insert and pET-21a vector was performed. Equimolar quantities of the insert and vector were heated to 70°C for 10 minutes and then cooled. To this mixture 4.3mM Tris-HCl, pH 7.5, 0.87mM $MgCl_2$, 0.87mM dithiothreitol, 87µmol L^{-1}, 2.17µg ml^{-1} bovine serum albumin and 87U ml^{-1} of T4 DNA ligase (New England BioLabs) were added and incubated at 16°C overnight. The ligated material was then transformed into *E. coli*.

Transformation into *E. coli*

Making Competent Cells:

Several colonies of *E. coli* in TG1 were inoculated into 10 ml of SOB medium. Growth at 37°C with rapid shaking took place for four hours. The culture was placed on ice for ten minutes. The cells were recovered by centrifuging at 3000 rpm for 10 minutes, at 4°C. The cell pellet was resuspended in 3ml of TFB and kept on ice for 15 minutes. This was centrifuged for 10 minutes at 3000 rpm at room temperature. The cell pellet was resuspended in 0.8ml of TFB. 28µl of DND was added and the mixture placed on ice for 10 minutes. After this another 28µl of DND was added and the mixture kept on ice for 10 minutes.

Transformation into Competent Cells:

The ligation mixture was pipetted into 1.5ml Eppendorf tubes and placed on ice. Competent cells (210µl) were added to each of the two Eppendorf tubes, one for the ligation mixture and one for the control (cut vector with no insert). The mixtures were left on ice for 30 minutes. After this time they were heat shocked for 115 seconds at 42°C and then returned to ice. The

final product (50µl) was spread onto ampicillin resistant LB agar plates and incubated at 37°C overnight.

Analysis of Colonies:

Following overnight incubation, colony counts were performed on both control and ligation mixture LB plates.

RESULTS

Analysis of the ISG-75 Sequence

Studying the basepair sequence of variant 1 was necessary to design appropriate primers in order to obtain the N-terminal domain. ISG-75 has three genetic sequence variations, for this project the variant 1 isoform was the desired product. Complimentary primers were designed based on the flanking sequences and used in the PCR. Appropriate restriction enzymes, Nde1 and Not1, were chosen based on this information. The primers designed were

_ Nde1 cuts here

5'-ggaattcca tatggaggagctctctgttgcg-3' and

_ Not1 cuts here

5'-actagagtggc ggccgctcacttcgttgtcccaactca-3'. The underlined sections correspond to the underline non-bold/italicized sections in Figure-1 respectively.

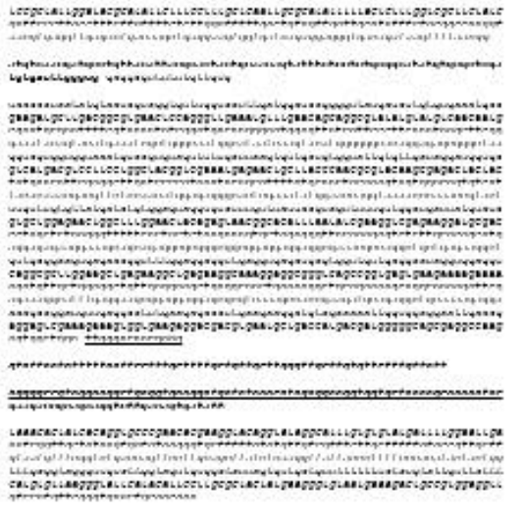

Figure-1: >ISG-75 variant-1 (genomic clone SW331), M86711,2162 BP[19]

In figure 1, each section is coded and separated for convenience. The italicized sections at each end of the sequence are noncoding regions. Underlined sections encode the leader sequence. Normal font text encodes the desired N-terminal domain. Bold and italicized section encodes the hydrophobic transmembrane domain. Bold, italicized and underlined section encodes the C-terminal domain. Underlined regions are the sequences that were used to construct the primers listed above.

PCR of the N-terminal from Genomic DNA of *T. brucei*

The expected band size of the N-terminal domain was 1354 basepairs. By comparing the band on the gel to a set of standards of known basepair size, it was found that the product of the PCR reaction was the correct size. The PCR product was cleaned, gel purified and the isolated product rerun on an agarose gel. Again, the DNA fragment encoding the N-terminal fragment was located in the expected region of the gel.

Restriction Digest of the N-terminal and pET-21a

After incubating the insert and vector with the restriction enzymes overnight, they were viewed using a long wavelength ultraviolet light source to prevent mutation. The N-terminal domain was visible in the appropriate region based on comparison with the standard. It should be noted that two smaller band fragments are also visible after this digestion. These fragments are the cleaved product of the ISG-75 variant 3 isoform. The restriction enzyme Nde1 cleaves this isoform into fragments 600 and 754 basepairs in length (see Figure 2). The pET-21a vector was also found in the appropriate region and measured 5443 basepairs in length.

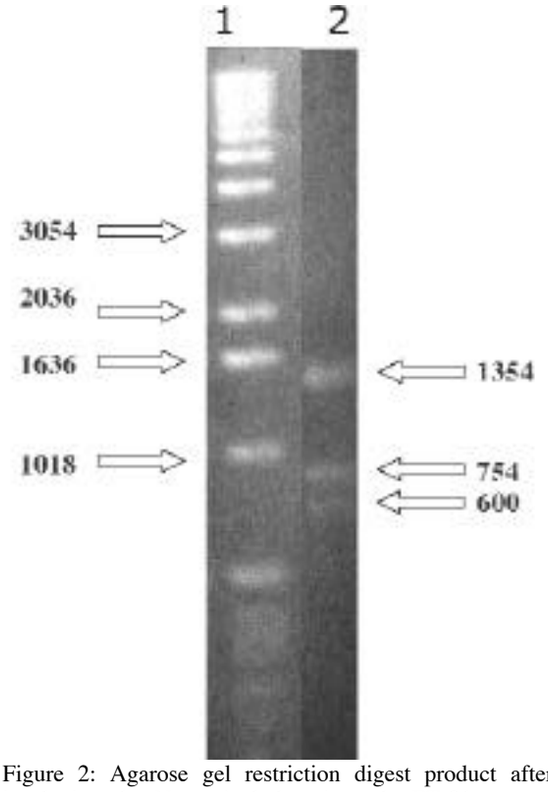

Figure 2: Agarose gel restriction digest product after incubating the N-terminal domain and pET-21a vector with restriction enzymes Nde1 and Not1. Column 1 is the control. Column 2 shows the digest products. Numbers listed are base pairs.

Transformation of Ligation Mixture into *E. coli*

E. coli colony counts where performed on both the ligation mixture and control LB agar plates. Thirteen colonies were found on the ligation mixture plate. No colonies were found on the control plate.

DISCUSSION

This experiment has provided an opportunity to learn and master many techniques in molecular biology. These include designing and running PCR, gel electrophoresis, gel extractions, ligations and transformations. A sound understanding and respect for these techniques will benefit a future medical career. These methods provide one of the modern foundations on which new drugs are developed and other therapeutic measures developed.

Other researchers will continue this project by subcloning the C-terminal domain. This 34 amino acid domain will be inserted into pGEX to produce a recombinant gene encoding a GST-fusion protein with a spacer arm linking the C-terminal domain to the GST. The purification of the C-terminal domain will be accomplished with a glutathione affinity resin.

After both domains have been subcloned the *E. coli* cell colonies of transformed material will be minipreped. The miniprep will be analyzed to ensure that the desired product has been obtained. Then the N-terminal domain will be purified using two ion exchange resins in tandem, DEAE-cellulose and CM-cellulose. Recent bioinformatic work has revealed that the 440 amino acid N-terminal domain has an isoelectric point of 5.17, using the protein characterization programme at the University of Marseille.[20] Consequently, at pH 5.17, this peptide should bind to neither DEAE nor CM cellulose, while essentially all other proteins will bind to one or the other of these exchangers, due to their charge at this pH. If necessary, a final gel filtration step can be included on either Biogel P-100 or Sephacryl S-300 to ensure complete purification.

SUMMARY

The goal of this project was to subclone the portion of the gene encoding the 440 amino acid N-terminal domain of *T. brucei* and transform it into *E. coli*. PCR was performed using genomic DNA to obtain the N-terminal domain of ISG-75. The gene fragment encoding the N-terminal domain of ISG-75 and the pET-21a vector were successfully double digested with the restriction enzymes, Nde1 and Not1. The insert and vector were cut from 1% agarose gels and extracted from the gel material. They were then ligated together and transformed into *E. coli*. The next part of this project will be to perform a similar procedure to obtain the gene fragment encoding the C-terminal domain. After this, both the N-terminal domain and the C-terminal domain will be expressed in *E. coli* and purified. These purified proteins will be used to raise antibodies for use in determining the orientation of ISG-75 in the plasma membrane of *T. brucei*. The orientation of this protein is important as a possible target for parasite control by chemotherapy or immune control via a single chain. This involves the production of a single domain recombinant antibody that would be small enough to pass the VSG barrier and reach its ISG target. Understanding which part of the protein is exposed externally on the cellular surface is critical in selecting the portion of the protein to target.

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Latent Tuberculosis is Highly Prevalent in Sub-Saharan Africans in Dublin - a Study Intended to Establish Normal CD4 Reference Ranges in this Population

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ABSTRACT
Objectives: To establish the normal reference range for CD4 lymphocyte cells in human immunodeficiency virus (HIV) negative sub-Saharan Africans attending the Genito-Urinary and Infectious Diseases (GUIDE) Outpatient Clinic at St. James's Hospital in Dublin. To correlate CD4 Count with lymphocyte count. **Design:** This was a prospective observational study. **Methods:** Volunteers were recruited among new sub-Saharan African patients attending the GUIDE Outpatient Clinic at St. James's Hospital, Dublin. Recruitment took place over an eight-week period between July and August 2003. The study objectives and methods were explained to volunteers, and informed consent for participation was obtained. History and relevant physical examination, together with measurement of haematological parameters, screening for sexually transmitted infections (STIs) and examination of stool and urine samples were performed to exclude confounding co-morbidities. A chest radiograph and Mantoux skin test was performed to exclude pulmonary tuberculosis (TB). **Results:** Seventeen participants were recruited. Two (12%) were excluded on the basis of HIV infection. Ten men and five women form the CD4 study group. Of the fifteen suitable patients recruited, the range of CD4 count is 532–1537 x 10^6/L. The reference range used by the laboratory at St. James's, based on CD4 counts in largely Caucasian populations, is 380 – 1500 x 10^6/L. Despite a high level of coincidental findings in the cohort, the range of CD4 counts measured falls within the range currently used by the laboratory. Women had significantly higher CD4 cell counts than men (median = 1245 and 899 respectively. $P < 0.01$), as has been previously described. There was a strong linear relationship between CD4 cell count and absolute lymphocyte count (r^2=0.5). Twelve of thirteen patients (92%) screened had evidence of latent pulmonary tuberculosis (TB), on the basis of a positive Mantoux reaction without radiographic evidence of TB. Two of fifteen (13%) HIV-negative patients defaulted before the result of their tuberculin skin test could be read. Only one patient in the entire cohort had a negative reaction to tuberculin challenge. We have referred 92% of this cohort for tuberculosis chemotherapy. **Conclusions:** The range of CD4 lymphocyte counts measured in this cohort falls within that used by the central pathology laboratory at St. James's Hospital, and by clinicians at GUIDE. This is the range that is used to guide clinical care among HIV-positive patients at GUIDE. A high rate of latent TB exists in this cohort.

INTRODUCTION

Tuberculosis (TB)

The World Health Organization estimates that one third of the world's population is infected with *Mycobacterium tuberculosis*, and that there are eight million new cases of active TB annually. Nearly 2 million persons die of TB worldwide each year, including persons infected with the human immunodeficiency virus (HIV).[1] The global incidence rate of TB is growing by approximately 0.4% per year, with a much faster growth rate in sub-Saharan Africa.[2] TB is currently responsible for approximately 11% of deaths occurring due to the acquired immunodeficiency syndrome (AIDS) worldwide. HIV is the single most important precipitant of the increased incidence of TB in Africa in the past 10 years.[1] TB is now the most common AIDS-defining illness in Africans resident in the UK.[3] The crude incidence rate of tuberculosis in Ireland fell in four consecutive years until 2000, where it was recorded at 10.9 cases per 100,000 population.[4] In the year 2000, 11.4% of cases of tuberculosis notified nationally were known to affect persons born outside Ireland.[5]

Background and Original Aims of the Study

International guidelines for the treatment of HIV infection have been drawn up using CD4 lymphocyte cell count reference ranges determined by studies conducted on HIV-negative North American and European subjects.[6,7] Several studies have shown that there is a significant difference in reference ranges between ethnic groups.[8,9,10] The primary aim of this study was to establish the normal reference range for CD4 cells in HIV-negative sub-Saharan Africans attending the Genito-Urinary and Infectious Diseases (GUIDE) Outpatient Clinic at St. James's Hospital, Dublin. This was a prospective observational study.

STUDY METHODS

General Considerations

Measurement of CD4 count at St. James's Hospital is undertaken as part of measurement of lymphocyte subsets. This is carried out by the department of immunology at the hospital's central pathology laboratory. Candidates for the study were drawn from new sub-Saharan African patients who registered with the GUIDE outpatient service over an eight week period between July and August 2003.

Consent, History-taking and Examination

Prior to participation, a detailed consent form was discussed with the patient, and informed consent was obtained for participation in the study. Demographic details and past medical and infection history were recorded, as were details pertaining to smoking preference. All patients underwent infectious disease screening in order to exclude ongoing infection. Participants had a general physical examination. Chest radiograph and tuberculin skin test (Mantoux method) were performed to identify tuberculosis. Stool samples were collected for culture and examination for ova, cysts and parasites. Optimal™ monoclonal antibody kits were used to screen for malaria. Urinalysis was performed to exclude pathological proteinuria, haematuria, glycosuria, and bacteruria (evidenced by the presence of nitrite in the urine), and of leucocyte esterase (indicative of the presence of white cells in the sample). Female patients underwent a pregnancy test based on urinary qualitative human chorionic gonadotrophin (βHCG) measurement.

A screen for sexually transmitted infection was performed. This included genital swabs for chlamydia, gonorrhoea, trichomoniasis and non-specific urethritis, and blood tests for HIV, hepatitis A, B and C, and syphilis. Absolute lymphocyte count was measured in each volunteer, together with lymphocyte subsets (including CD4 count).

Statistical Analysis

Statistical analysis of CD4 lymphocyte counts was undertaken using the Statistical Program for the Social Sciences (SPSS). The range of a group of CD4 cell counts is positively skewed. "Skew" or "bias" is used to describe a distribution that is not normal. This reflects the fact that although the upper limit of a range of CD4 cell counts may be very high, the lower limit cannot be less than zero.

Because of this positive skew, standard statistical tests based on normal distributions such as the Student's T-test are weakened. Non-parametric analysis does not require that variables under examination must be distributed normally about the mean of the sample. This is the reason that non-parametric analysis is more appropriate to a sample consisting of CD4 cell counts than a simple parametric test such as the t-test. The Kruskal-Wallis rank sum test used in this instance is a non-parametric statistical test.

The coefficient of determination r^2 was calculated as a test of the strength of the correlation between CD4 Count and absolute lymphocyte count. For two given variables *x* and *y* measured in a sample, r^2 represents the proportion of the variability of *y* that can be attributed to its linear relationship with *x*, where r is the Pearson product moment correlation coefficient "correlation coefficient". In this study, r^2 is quoted as a measure of the strength of the relationship between CD4 count and absolute lymphocyte count.

RESULTS

Seventeen new patients of sub-Saharan origin were recruited over an eight week period between July and August, 2003. Eleven men and six women were enrolled during this time. The original intention of the study was to establish a range for CD4 lymphocytes in sub-Saharan African patients.

Recruitment was hampered by a sharp decline in the number of new patients from sub-Saharan Africa attending GUIDE outpatient clinics during the study period. Most new patients from sub-Saharan Africa at GUIDE are asylum seekers who are in the process of applying for refugee status in this country. This curtailment of numbers may reflect a national trend in the rate at which people sought asylum in Ireland during 2003 (figure 1).

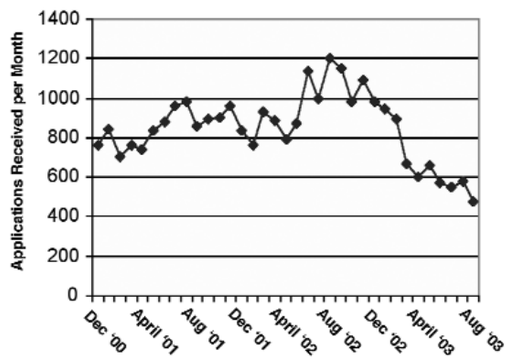

Figure 1: Applications for asylum received by the Refugee Applications Commissioner's Office between December 2000 and October 2003.[11]

Seventeen volunteers from eight countries in sub-Saharan Africa were recruited. The majority of our volunteers were born in

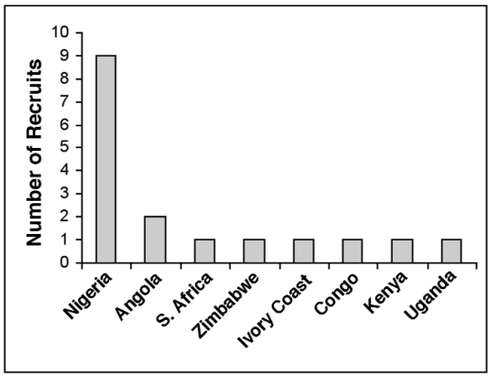

Figure 2: Country of origin of study recruits (N=17).

Nigeria (N=9). Two were born in Angola, and one in each of the other countries listed in figure 2.

Two of our seventeen original volunteers (12%) were diagnosed HIV-positive, a Nigerian woman and a man from the Ivory Coast. These patients were excluded from the CD4 study calculations. The range of CD4 lymphocyte counts in the fifteen suitable patients recruited is 532 – 1537 x 10^6/L. The reference range used by our laboratory, based on CD4 counts in largely Caucasian populations, is 380 – 1500 x 10^6/L.

The range of CD4 lymphocyte cell counts measured in our cohort falls within the range

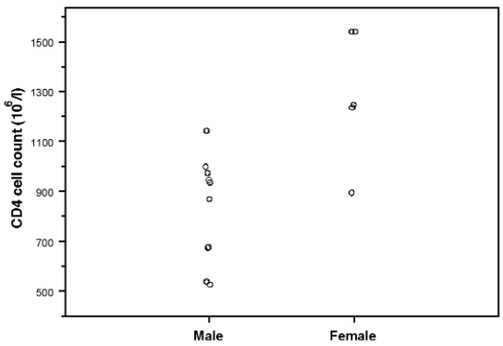

Figure 3: Sex variation in CD4 count.

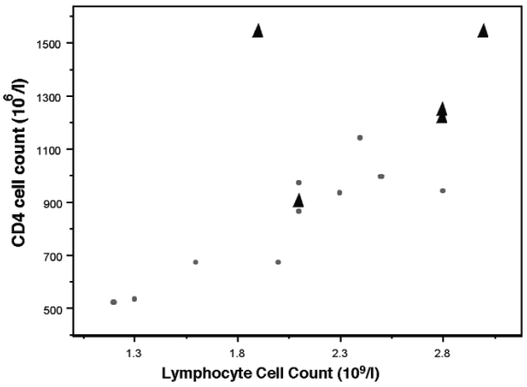

Figure 4: CD4 and lymphocyte counts in men (●) and women (▲).

currently used by our laboratory. Women had significantly higher CD4 cell counts than men (median = 1245 and 899 respectively. $P < 0.01$), as has been previously described (figure 3).[4] This is based on a Kruskal-Wallis rank sum test.

Smoking is known to affect CD4 count. Smokers are known to have significantly higher CD4 counts than non smokers.[12] Three (20%) of the patients in this cohort were smokers. No difference was found between the CD4 counts of smokers and non-smokers in this small group. There was a strong linear relationship between CD4 cell count and absolute lymphocyte count (r^2=0.5) (See figure 4). This value indicates that at least 50% of the variation in CD4 is explained by the variation in lymphocyte count.

In order to establish that the range of CD4 lymphocyte counts in our cohort was valid as a reference range in the wider population, it was important for us to exclude infective causes of altered lymphocyte count in the study cohort. Table 1 details potentially confounding pathologies identified in members of the study group. Despite the wide range of incidental pathologies identified in the study group, measurement of CD4 count lies within the range currently used by the hospital.

Table 1: A range of pathologies identified in the cohort.

Pathology detected	Number of patients
Cysts of *Endolimax nana**	2
Cysts of *Entamoeba histolytica/dispar***	1
Chronic anemia of unknown origin	1
Active Hepatitis B infection	1
Latent syphilis	1
Chlamydia trachomatis, Trichomonas vaginalis , Bacterial vaginosis	1
Non-specific urethritis	1

* Evidence of inactive disease ** Morphologically identical cysts

Latent TB in the Study Group

No member of this study group had radiographic evidence of infection with TB (N=15). Twelve of thirteen patients successfully screened (92%) had evidence of latent TB, on the basis of positive Mantoux reactions of between 12 and 30 mm induration. Two patients defaulted before the result of their tuberculin test could be read. Only one patient had a negative reaction to tuberculin challenge (see figure 5).

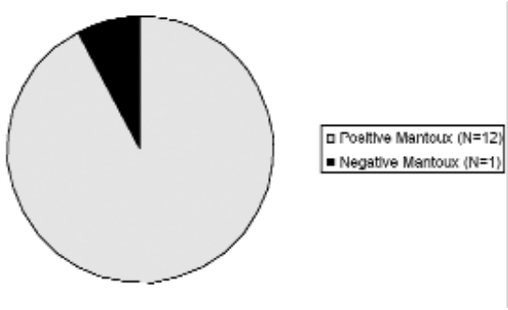

Figure 5: Latent TB infection in screened members of study group (N=13).

DISCUSSION

CD4 Count and the Establishment of a Reference Range

Attempts to establish a reference range for CD4 lymphocyte counts in sub-Saharan Africans were hampered by a low rate of recruitment, as outlined previously (see figure 1). CD4 lymphocyte counts are used to monitor the progression of HIV infection, and to determine intervention thresholds at which highly active antiretroviral therapy (HAART), and antibiotic therapeutic prophylaxis are initiated. In this study, CD4 counts measured fell within the reference range used at GUIDE.

A strong linear relationship was found between CD4 count and absolute lymphocyte count in this study group. Were it to be carried on into a larger group, this would support the use of absolute lymphocyte count as a surrogate marker for CD4 count. Such a finding has implications for the provision of health services in the resource-limited setting of the developing world. It is very much less expensive to measure absolute lymphocyte count than CD4 count. The ability to monitor HIV progression by measuring absolute lymphocyte count would reduce the cost of providing care for HIV-positive patients. More studies are needed in this area.

Other Pathologies in the Cohort

We identified a range of other pathologies in our cohort, including a range of sexually transmitted infections. An untreated sexually transmitted infection increases the likelihood of transmission or acquisition of HIV infection by six- to tenfold. A genital ulcer is thought to increase the risk of becoming infected with HIV during a single exposure by up to 300-fold.[13]

During the initial history taking phase of the study, each member of the cohort was explicitly questioned about relevant infections in their past. No member of this cohort volunteered a history of any of the illnesses with which they were subsequently diagnosed. One must therefore surmise either that existing screening provisions failed to diagnose their conditions at point of entry, or that their conditions were contracted in the interval between their arrival in Ireland, and presentation at GUIDE.

Latent TB in the Study Cohort

According to the current TB statistics published by World Health Organization (WHO), all members of our cohort come from countries that the British Thoracic Society (BTS) define as "high risk" for TB (incidence of 40 per 100,000 population or greater).[2,14]

Thirteen of fifteen members (87%) of the HIV-negative cohort were successfully screened by Mantoux skin testing. In this cohort, twelve of thirteen (92%) of the HIV-negative sub-Saharan African volunteers who were successfully screened had latent tuberculosis. Only one of the thirteen candidates who were successfully screened tested "negative" for TB in this instance.

We have referred 92% of the screened group for prophylactic tuberculosis chemotherapy. Two of fifteen members (13%) of the original cohort defaulted before the results of their Mantoux test could be read. The two-stage process required for Mantoux testing reduced its efficacy as a screening intervention in this case.

Two of the seventeen original recruits were diagnosed HIV-positive. One HIV-positive candidate had an anergic reaction to tuberculin. The other HIV-positive patient had a positive Mantoux reaction, and has received TB chemotherapy at GUIDE on this basis.

Current recommendations for chemoprophylactic treatment of latent tuberculosis in Ireland are that the patient should receive isoniazid for 6 months, and for at least 9 months if they are HIV-positive. Severe hepatotoxicity and death have been reported with protracted isoniazid treatment regimes.[15] Because of the risk of peripheral neuropathy in patients receiving isoniazid, it is prudent to prescribe pyridoxine (vitamin B_6) at a dose of 10mg daily from the start of treatment.[16]

Although it is administered only once daily, rates of non-compliance of between 24%

and 28.5% have been reported in patients receiving isoniazid monotherapy for latent TB.[17,18] Ireland has not yet implemented the directly observed treatment short-course (DOTS) endorsed by the WHO at a national level.[2] DOTS allows the documentation of all doses received by a patient during chemotherapy, and requires the direct observation of the patient as they ingest their medicine.[19] The DOTS strategy was first devised to address the resurgence of TB in the context of the emergence of drug resistant TB.

Since the beginning of the new century, drug resistant tuberculosis has emerged in Ireland. Between 2000 and 2001, the number of cases of multi-drug resistant TB notified in Ireland almost trebled. In 2001, in the Eastern Reional Health Authority area alone, eight reported cases of tuberculosis were resistant to one or more antibiotic. Five of eight cases occurred in non-national persons.[4]

TB is a notifiable disease in Ireland, under the provision of the 1947 Health Act. Provision under the act is made for an individual who refuses to cooperate with therapy to be confined to a hospital in order to receive therapy. It is unclear whether this provision has ever been implemented. All aspects of the management of TB are entirely free to patients. This includes hospital care and all medications, which are provided through the local Health Board.

International TB Screening Recommendations

The diagnosis of latent TB is made on the basis of a tuberculin skin test and a normal chest radiograph.[15] The American Thoracic Society and the Centers for Disease Control and Prevention (CDC) classify persons with a positive tuberculin skin reaction, but no clinical, bacteriological or radiographic evidence of active tuberculosis, as having latent tuberculosis.[20] No studies have been carried out on the treatment of latent TB in Ireland. Prophylactic treatment of latent TB has a definite but variable benefit. Almost all clinical studies have used the Mantoux method of tuberculin skin testing. This is the only technique recommended by the World Health Organization and by the International Union against Tuberculosis and Lung Disease.[15]

Mantoux testing involves the intra-dermal injection of 0.1ml of purified protein derivative in the anterior forearm. The result, read 48 – 72 hours later, is recorded as the diameter in millimetres measured transversely to the long axis of the forearm. Only induration or thickening in the skin is recorded; redness or localised oedema is ignored. In Ireland, a reaction of 10mm is commonly taken as evidence of sensitization to the infection.[15]

Where close contact has occurred with individuals who have infectious tuberculosis, there is a 25 – 50% chance of being infected with *Mycobacterium tuberculosis*. Infection rates are similarly high among adults in countries where tuberculosis is very prevalent. In such cases, the specificity of the tuberculin test is very high, and a positive tuberculin skin test indicates a high likelihood of tuberculosis infection.[20] BTS guidelines recommend the following in patients with strongly positive reactions to tuberculin: "chemoprophylaxis is recommended for those with a history of contact with infectious tuberculosis or residence in a high prevalence area within the preceding two years".[14]

The CDC recommend that prophylactic TB therapy is considered for HIV-positive individuals who have a Mantoux reaction with induration greater than 5mm, or in those who fail to react to tuberculin. These guidelines are offered regardless of whether the individual has received Bacillus Calmette Guerin vaccination in the past.[21] It is important to note that American Mantoux testing is based on a preparation of tuberculin that is more biologically potent than the preparation used in Irish testing (5TU as opposed to 2TU tuberculin). This is one reason for the lower threshold of diagnosis (5mm compared to 10mm induration).

Efficacy of BCG Vaccination in Preventing TB

The current recommendations of the CDC conclude that a positive reaction to tuberculin challenge subsequent to BCG vaccination cannot predict whether any protection against the disease has been acquired.[22] The protective efficacy of the vaccine is known to diminish over time. The largest study of its kind demonstrated no protection from BCG vaccination against infection by *Mycobacterium tuberculosis* in adults or children 5 years after initial vaccination.[23] Also, no evidence exists of the protective value of the vaccine in patients subsequently infected with HIV.[22]

Four of the thirteen successfully screened members of our cohort (30%) had evidence of BCG vaccination, while 92% had latent TB. This serves to reiterate that vaccination with BCG does not confer universal immunity to TB.

The Ethics and Legalities of Screening Immigrants

The International Health Regulations endorsed by the WHO state that it is illegal to for a nation to require proof of health, or to order the compulsory screening of immigrants on health grounds prior to their arrival in the country.[24]

The United States requires screening for Tuberculosis as a prerequisite for visa applicants.[21] The BTS recommend screening of new entrants to the United Kingdom from high risk areas of the world (TB incidence of more than 40 per 100,000 population per year), and of all refugees.[14] This is a statutory regulation in the UK.

CONCLUSIONS

The range of CD4 lymphocyte counts measured in this cohort falls within that used by the central pathology laboratory at St. James's Hospital, and by clinicians at GUIDE. This is the range that is used to guide clinical care among HIV-positive patients at GUIDE. A high rate of latent TB exists in this cohort.

The findings of this study raise questions about the efficacy of screening for TB in this cohort of foreign born persons. It is important that strategies be found to increase the uptake rate and successful completion of voluntary screening for TB in vulnerable groups from high-risk areas of the world.

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CONFLICT OF INTEREST STATEMENT

No conflicting interests are declared.

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Prescription of Combined HRT in Non-Hysterectomised Women in General Practice

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ABSTRACT
Objective: The prescribing practice of combined hormone replacement therapy (HRT) medication in non-hysterectomised women in one Dublin general practice (GP) surgery from 1996 to 2003 was investigated in this study. The aim of the study was to explore whether Irish GP prescribing practice reflected the Irish College of General Practitioners (ICGP) guidelines from the Saffron Initiative (referenced in the Monthly Index of Medical Specialties (MIMS)) which guide the timing of prescription of combined cyclical HRT (CYHRT) versus continuous combined HRT (CCHRT) according to age. **Methods:** Using the GP Clinical Computer program in the practice, the number of prescriptions of combined HRT, the age of women at the time of their first prescription, the average age of prescription of CYHRT versus CCHRT and the percentage of women older or younger than the recommended ages for each type of combined HRT were calculated. **Results:** A total of 90 prescriptions for CYHRT were identified during the time period 1996 to 2003. The age range of the women was 40 to 57 years with an average age of prescription of 49.73 years. It was found that 24% of the prescriptions made were to women above the recommended cut-off point of 51 years. A total of 60 prescriptions for CCHRT were found for the time period 1996 to 2003. The age range of these women was 44 to 61 years with the average age of prescription being 51.33 years, of whom 32% were below 51 years. **Conclusion:** The age cut-off point of 51 years applied to most patients in terms of prescribing CYHRT versus CCHRT. It did not apply to all patients though and this fact is in keeping with updated ICGP guidelines from the Saffron Initiative. This emphasises the importance of evaluating each patient on an individual basis.

INTRODUCTION

The menopause commonly occurs between the ages of 45 and 55 years with a mean age of 51 years. This onset has not changed for at least two centuries and appears to be unrelated to age at menarche, socioeconomic factors or body mass index.[1] Menopause means cessation of menstruation and is said to have commenced when a woman has been amenorrhoeic for 12 months. The menopause is caused by ovarian failure. Ovarian failure can produce a range of symptoms that affect both the quality of life and well-being of women of this age. Hormone replacement therapy (HRT) has long been used to address these symptoms.

HRT generally implies oestrogen-based therapy (with or without additional progestogen). In non-hysterectomised women, combined HRT preparations are preferred as the additional progestogen has a protective effect on the endometrium. These are divided into cyclical (CYHRT) and continuous combined HRT (CCHRT) preparations.

During the early stages of the perimenopausal period women commonly experience irregular and/or heavy vaginal bleeding. Vaginal bleeding has been cited as the most frequently reported reason for stopping HRT with approximately one-third of women stopping it for this reason.[2,3]

Cyclical HRT often helps combat the perimenopausal symptom of irregular bleeding.[4] Irregular bleeding has been shown to occur more frequently with CCHRT than with CYHRT during the first 6 months of follow-up. However, this risk diminishes over time and amenorrhoea is normally achieved within one year in those taking CCHRT. Overall, women have approximately a 1 in 3 chance of incurring irregular bleeding in the first few years of starting either CCHRT or CYHRT.[5]

The decision to commence HRT is becoming more and more complicated both for patients and medical practitioners alike. Long-term health safety concerns battle with quality of life issues in this risk-benefit analysis. The latest research in this area shows a significant increased risk of breast cancer with only 1-2 years use of combined HRT and a potential cardiovascular risk during the first year of use of HRT.[6]

The Chairman of the Committee for the Safety of Medicines (CSM) in the UK has been recently quoted as saying "the decision to start HRT is individual for each patient and should be re-assessed annually". The CSM itself reports that "for short-term use of HRT for the relief of menopausal symptoms, the benefits outweigh the risks for many women".[7]

GPs are the initiators of approximately 84% of all HRT prescriptions.[8] HRT is generally thought to be under-prescribed and even when issued, compliance with long-term therapy is

relatively poor. Less than 50% of women prescribed HRT are using it after nine months.[2]

In Ireland, the Saffron Initiative, which takes its name from the late flowering Saffron Crocus, was established in 1997 with a view to identifying opportunities for improving the health of women in the second half of their lives. The Saffron Initiative Steering Committee was comprised of a multidisciplinary highly experienced group of members in the field of women's health.[9]

The Saffron Initiative published guidelines in 2000 for GPs which recommended that non-hysterectomised women below the age of 51 years or those who were less than one year postmenopausal should be prescribed CYHRT. It recommended that non-hysterectomised women over 51 years or those at least one year postmenopausal should be prescribed CCHRT.[10] The rationale behind this was to minimise the potential for irregular vaginal bleeding. This information is published along with an explanatory flow chart (figure 1) in current editions of the Monthly Index of Medical Specialties (Ireland) (MIMS).[11] The British National Formulary (BNF) gives similar advice without using an age cut-off point. It recommends that CCHRT preparations should not be used by women who are perimenopausal or those within 12 months of their last menstrual period.[12]

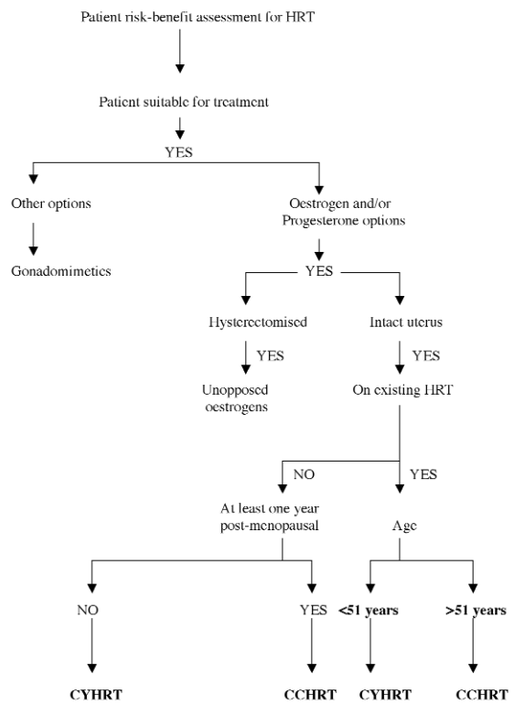

Figure 1 : HRT – Guide to Selection
Source : MIMS Ireland July 2003[11]

The aim of this study was to explore whether Irish GP prescribing reflected recommended Irish guidelines in relation to the timing of CYHRT and CCHRT prescriptions during the time period 1996-2003.

METHODS

The research was carried out as part of a two-week GP summer elective. Access to the GP Clinical Computer system of the busy three-doctor practice in Dublin was granted and a review of combined HRT prescriptions from 1996 (when the computer system was introduced in the practice) until July 2003 was performed.

The GP Clinical Computer system contained data on all patients (clinical records and medications prescribed) from 1996. The July 2003 edition of MIMS provided the list of CYHRT and CCHRT medications on the market for some or all of the study period (table 1 and table 2).[11]

Criteria for inclusion in the study were women with intact uteri who were prescribed the combined HRT medication whose starting date for the medication was clear from the records. Criteria for exclusion were hysterectomised women and women prescribed the medication with insufficient data (on the computer system or from written medical notes) as to their exact starting date on the medication.

For the purposes of this study, each medication listed in table 1 and table 2 was entered in turn into the GP Clinical Computer Programme. This provided a list of patients to whom the particular form of CYHRT or CCHRT had been prescribed since 1996. Each individual patient's computerised notes were subsequently investigated to find out the first date the HRT had been prescribed and the age of the woman at that date. Any alterations in HRT medication (and the age of the woman with each alteration) were also noted. If a woman was prescribed different forms of HRT, each prescription was counted as a separate entry in terms of data collection. In cases where there was a lack of clarity regarding the age of the woman at the start of her combined HRT or where she had already started HRT, old written medical case notes were accessed and checked for this information.

The recommendations used to assess the prescribing practice in this study were published for the attention of Irish GPs by the Saffron Initiative in March 2000 and this investigation examined records back as far as 1996. Subsequent to March 2000, monthly editions of MIMS produced a flow chart (figure 1) as well as written guidelines detailing the recommended prescription of combined HRT.[11] On close examination of the flow chart published monthly

Table 1. MIMS "July 2003" List of CYHRT.

Trade name	Oestrogen	Progesterone	Formulation
Estracombi	Oestradiol 50mcg/day	Norethisterone 250mcg/day	Transdermal patches
Estrapak	Oestradiol 50mcg/day	Norethisterone 1mg	Patch + oral tablet
Estalis Sequi	Estradiol 50mcg/day	Norethisterone 250mcg/day	Transdermal matrix patches
Femoston 2/10	Oestradiol 2mg	Dydrogesterone 10mg	Oral tablet
Femoston 2/20	Oestradiol 2mg	Dydrogesterone 20mg	Oral tablet
Nuvelle	Oestradiol 2mg	Levonorgestrel 75mcg	Oral tablet
Premique Cycle 10	Conjugated oestrogen 0.625mg	Medroxyprogesterone 10mg	Oral tablet
Prempak C	Conjugated oestrogens 0.625 or 1.25mgs	Norgestrel 0.15mg	Oral tablet
Trisequens	Oestradiol 2mg/1mg Oestradiol 4mg/1mg	Norethisterone 1mg Norethisterone 1mg	Oral tablet
Trisequens Forte			Oral tablet

in MIMS (figure 1) it is seen that the age recommendation using 51 years as the cut off applied to women on existing HRT treatment. For those women starting treatment for the first time it was recommended to start CYHRT only if they were less than one year postmenopausal. For the purposes of this study this was taken also to mean that they were 51 years or younger as the mean age for the menopause is 51 years. Thus all women whether newly prescribed HRT or those with prior experience of HRT medication had 51 years of age as their cut-off point between CYHRT and CCHRT.

The number of prescriptions of combined HRT, the age of women at the time of their first prescription, the average age of prescription of CYHRT versus CCHRT and the percentage of women older or younger than the cut-off age of 51 years were analysed in this study.

RESULTS

There were 9112 patients registered on the computer in this general practice. This figure was estimated to account for over 95% of the practice population. Of the 9112 patients, 5057 were female and 4055 were male. Of the females, 2327 were over 40 years of age.

From the records, 90 prescriptions for CYHRT were identified during the time period from 1996 to 2003. The age range of these women

Table 2. Taken from MIMS "July 2003" list of CCHRT

Trade name	Oestrogen	Progesterone	Formulation
Activelle	Estradiol 1mg	Norethisterone 0.5mg	Oral tablet
Estalis	Estradiol 50mcg	Norethisterone 250mcg/day	Transdermal matrix patch
Evorel Conti	Oestradiol 50mcg/day	Norethisterone 170mcg/day Dydrogesterone 5mg	Transdermal patch
Femoston-Conti 1/5	Oestradiol 1mg	Medroxyprogesterone 2.5mg/5mg	Oral tablet
Indivina	Oestradiol 1mg/2mg	Norethisterone 1mg	Oral tablet
Kliogest	Oestradiol 2mg	Norethisterone 1mg	Oral tablet
Novofem	Estradiol 1mg Conjugated oestrogens	Medroxyprogesterone 5mg	Oral tablet
Premique 5	0.625mg		Oral tablet

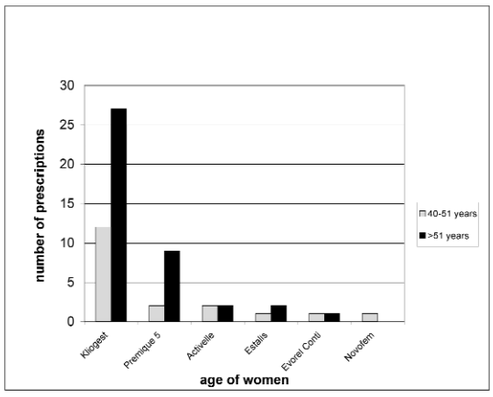

Figure 2: Number of prescriptions of CYHRT related to age at first prescription

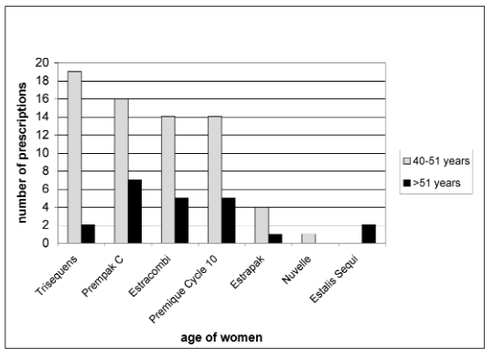

Figure 3: Number of prescriptions of CCHRT related to age at first prescription

was from 40 to 57 years. Despite the average age of prescription of this medication being 49.73 years, it was found that 24% of the prescriptions made were to women above the age of 51 years (figure 2).

Only 60 prescriptions for CCHRT were identified during the time period from 1996 to 2003. The age range of these women was from 44 to 61 years with the average age of prescription being 51.33 years. Of these, 32% of these women were below the age of 51 years on starting this medication (figure 3).

DISCUSSION

This study investigated the age-appropriateness of HRT prescription in non-hysterectomised women in one Dublin GP surgery. It found that the majority of prescriptions were appropriate and in keeping with the recommended guidelines. Approximately one in four women who were receiving CYHRT were being prescribed it above the age of 51 years, which may not be as effective as CCHRT prescription above this age. Of the women receiving CCHRT, approximately one in three were being prescribed it below the age of 51 years and as a result were possibly at an increased risk of unwelcome side effects such as breakthrough bleeding. Of course, not all women reach the menopause at the age specified in the guidelines. Some of the women in these groups may have had premature or late menopausal symptoms and thus the prescriptions used may have been clinically accurate. Thus the findings from this study suggest that GPs are assessing their patients on an individual basis and are not basing their prescribing decisions on a flow chart which may not adequately demonstrate the age diversity of menopausal onset.

Although HRT is generally perceived to improve a menopausal woman's quality of life in the short term, recent evidence shows that this may not be the case. Not only are women at a possible increased risk of cardiovascular health problems and breast cancer when starting treatment but they may also be at risk of disruptive side effects such as irregular vaginal bleeding from HRT.[6,13]

A European study of 1871 women examined women's expectations of HRT. Cultural differences were apparent. Women in Germany and France were more unwilling to take HRT because of perceived contraindications compared to women in the UK (23% and 20% respectively versus 10%). Fear of side effects was highest in Germany (20%), followed by the UK (14%) and France (8%). The European study found that 72% of the women questioned were not taking HRT, mainly because it had not been suggested or recommended to them by their prescribing physician.[3] By comparison an Irish study found that 78% of Irish GPs would offer HRT to all eligible women.[14]

A survey of British women's views on the menopause and HRT found that 31% of women discontinued HRT within 6 months, 51% within 12 months and 74% within 3 years.[8] Withdrawal bleeding, lack of symptom control, oestrogenic and progestogenic side effects were the specific reasons for discontinuation in the majority of cases. Results from this British study suggest that women who were started on CYHRT in this study may have had side effects from this medication (possibly irregular bleeding) and thus were unwilling to continue with the medication. This may be relevant to the current study in light of the fact that 95 prescriptions were written for CYHRT and only 60 prescriptions for CCHRT. Irregular bleeding is a major cause for concern amongst postmenopausal women however it need only be investigated if it persists for longer than one year while on treatment.[13]

Less than 20% of peri- and post-menopausal women in the UK are currently taking HRT.[15]A survey of 600 Irish GPs published in 2000 found an average estimated prescribing rate

of HRT of 17.5% (interquartile range 10% to 30%).[14] The prescribing rate of HRT was not determined in the current study as it focused on combined HRT only. Any figure calculated would not be representative of total HRT prescriptions as oestrogen only preparations were not investigated.

The reason that figures from 1996 onwards were used in this study is that the research was limited to one general practice surgery with a patient population of approximately 9112 patients. Therefore, in order for this retrospective study to be meaningful in terms of patient numbers, it was deemed necessary to look at all cases from 1996 when the GP Clinical Computer Programme was introduced at the practice. This approach was felt to be both justifiable and appropriate as this study did not aim to conduct a strict statistical analysis. Rather this study looked at prescribing practice over a meaningful period of time and compared and contrasted this with guidelines which continue to be published in MIMS. It is pertinent to note that only 5 out of the 18 combined HRT drugs listed in MIMS 2003 were listed in MIMS 1996. There were also two other combined HRT medications listed in MIMS 1996 which are not in current use.[11,16] Thus, a retrospective study involving patients from more recent years could have yielded different results particularly as the Saffron Initiative guidelines were only published for the first time in the year 2000.[10]

It is interesting to note that the Saffron Initiative updated the GP guidelines in 2002 to reflect current knowledge about HRT in terms of benefit and risk. As part of that update, the recommendations regarding age cut-off were altered. The Saffron Initiative guidelines to GPs now recommend that only when a woman's periods become lighter on CYHRT or she is "approaching the ages of 53 or 54" should she change from CYHRT to CCHRT.[17] They thus have relaxed their guidelines and are no longer employing a strict age cut-off point to guide clinical decision making. This is more in keeping with what the BNF recommends and with current research findings.[12,13] Of note, these new Saffron guidelines have not yet been referenced in MIMS. It is still publishing the Saffron guidelines from the year 2000.[11]

Limitations of the study were lack of time to investigate reasons for combined HRT prescription, the length of time these women were compliant with these various forms of HRT, their incidence of side effects (including irregular bleeding) and their reasons for stopping the treatment. This information would have been particularly interesting in relation to those women whose prescription was not in keeping with the recommended age guidelines.

CONCLUSION

This study investigated the age-appropriateness of combined HRT prescriptions in non-hysterectomised women according to MIMS published guidelines. It showed that while the age cut-off point of 51 years applied to most patients, it may not suit all patients and this in fact, is in keeping with updated ICGP guidelines from the Saffron Initiative.[16] This finding emphasises the importance of evaluating each patient on an individual basis as has been recently recommended by the National Medical Informations Centre.[18]

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A Case of Friedreich's Ataxia

Aisling Snow
6th year Medicine

INTRODUCTION

M is a sixteen year old girl admitted electively for multidisciplinary review of Friedreich's Ataxia (FA), hypertrophic obstructive cardiomyopathy (HOCM), kyphoscoliosis and peripheral neuropathy. The following history was obtained from M, with collateral information from her father.

HISTORY OF THE PRESENTING COMPLAINT

M's parents and teachers noticed progressive clumsiness and stumbling from the age of six years and a diagnosis of FA was made on the basis of 'nerve tests' in 1994 when she was seven. M was then referred to a Dublin paediatric hospital for confirmation of the diagnosis and further specialist investigation.

M began using a wheelchair when she was eight and has used it full-time since the age of ten. In 2001 M obtained an electric wheelchair, which she now uses most of the time. She cannot stand, walk, or transfer from her wheelchair unaided. M is left-handed but over last two months has begun to find it difficult to feed herself and guide the electric wheelchair due to weakness & loss of co-ordination in her left arm.

Upon referral to Dublin in 1994, M was diagnosed with HOCM and now suffers from periodic 'muscular' chest pain. Although she currently has no chest pain, her last episode was one week prior to admission. She is always breathless on exertion, but never at rest.

M's kyphoscoliosis has been progressive since the age of eleven but does not restrict her breathing.

Both of M's lower limbs are affected by a peripheral sensory neuropathy, the left more so than the right. M has a good foot care regime, assisted by her parents.

PAST MEDICAL HISTORY

M has Type 1 diabetes mellitus (Type 1 DM) which was diagnosed at the age of four years. She currently uses Humalog insulin via subcutaneous pump. M also has hypothyroidism, which was diagnosed at the age of 14 years and is asymptomatic on thyroxine replacement therapy.

M has presented to A&E with diabetic ketoacidosis (DKA) on three occasions , twice in 2002 and once in February 2003. She was subsequently admitted twice for regulation of blood glucose levels. M was also admitted to hospital for three days in early 2003 with chest pain; her parents were told it was 'muscular'.

M has suffered multiple fractures since late childhood, none of which have required admission or surgery. All involved falls due to ataxia.

BIRTH, DEVELOPMENTAL AND SCHOOL HISTORY

M's mother had an uneventful pregnancy and she was born at full term weighing 8lb 6oz (3.78kg). Delivery was by caesarean section due to foetal distress during labour. M was not admitted to the special-care baby unit and went home with her mother after three days.

M walked at thirteen months of age and her father considers her development to have been normal. She progressed normally at preschool and in junior and senior infants, having no gross motor, fine motor or speech and hearing difficulties.

M is now in her fifth year in a mainstream secondary school. Her mother brings her to and collects her from school every day. M is absent from school for approximately 3-4 weeks per term due to illness and hospital admissions. She has many friends at school and socialises with them every weekend.

NUTRITIONAL, IMMUNISATION AND SOCIAL HISTORY

M was breast fed and was introduced to solids at the age of six months. She now eats a diabetic diet but does not use the diabetic exchange method as she has a subcutaneous insulin pump in situ. M's father cannot remember all the immunisations that she received but does not recall any being missed. She remembers receiving the rubella vaccine during her second year at post-primary school.

M's parents are married and live with M and her sister. Her father does not work and her mother is a factory supervisor. Neither parent smokes. The family lives in a bungalow that has been adapted to facilitate M's wheelchair.

M does not smoke. She drinks approximately 4 units of alcohol at the weekend but does not use recreational drugs.

FAMILY HISTORY & GENETICS

Both a maternal and a paternal aunt have Type 1 DM, as does M's younger sister. A paternal aunt has cerebral palsy. All maternal and paternal aunts and uncles underwent chromosomal karyotyping to determine whether they were carriers of the abnormal gene responsible for Friedreich's Ataxia. None were found to carry the

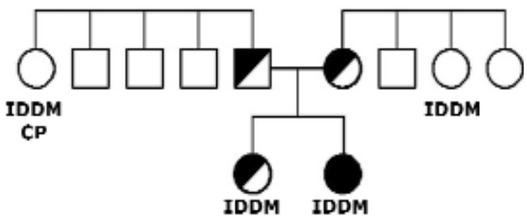

Figure 1: M's Family Pedigree.

gene.

All immediate family members have received extensive genetic counselling and M's sister is aware that if she decides to have children her partner's karyotype should be tested to ascertain whether he too is a carrier of the FA gene. There is no family history of cerebrovascular, cardiac or thyroid disease.

DRUG HISTORY

M is on Humalog insulin that is delivered via a subcutaneous pump system. The line is changed every three days by M and her parents and the dose is adjusted in accordance with dietary intake, exercise and infection. M also takes Eltroxin (thyroxine) 200mg nocte and Brufen (ibuprofen) as required (for pain associated with kyphoscoliosis). She takes no non-prescription drugs and has no known drug allergies.

SYSTEMS REVIEW

At the time of the history M felt well, with a good appetite and no recent weight loss. She had had no recent headaches, faints or double vision and has never had a seizure.

M suffers from periodic chest pain. The latest episode was one week prior to admission, lasted a day and was similar in nature to the pain which prompted her admission to hospital earlier this year. She is always breathless on exertion but not at rest and at the time had no other upper or lower respiratory tract symptoms, dizziness or palpitations.

M stated that her hips click painfully when getting in and out of her wheelchair or her bed. In addition, her kyphoscoliosis causes pain on prolonged sitting. M attends physiotherapy once a week.

Although M is continent of both urine and faeces she requires assistance in getting from her wheelchair to the toilet. She did not complain of abdominal pain, vomiting, diarrhoea or constipation and has no frequency, urgency or dysuria. Her menarche was at the age of thirteen and she now has a regular twenty eight day cycle with bleeding for four days.

EXAMINATION

M measured 170cm in height and weighed 65.2kg. Pulse rate was 73 bpm, respiratory rate was 16, blood pressure was 119/76 mmHg and temperature was 39.6^{o}C. She looked well and was alert, orientated and sitting up in bed. Her upper body movements were ataxic and her speech was dysarthric.

Cranial nerve examination was normal except for bilateral jerky horizontal nystagmus. In the upper limbs there was bilateral pyramidal drift. There was wasting of the small muscles of both hands and of the proximal muscles of the left arm. Tone was normal bilaterally. There was slight weakness of the left arm (grade 3-4 out of 5). Power in the right arm was good (grade 4 out of 5). Light touch sensation was normal in all dermatomes (C4 to T2) with no pain or tingling. Vibration sense was normal and there was normal proprioception at the distal interphalangeal joint of both thumbs. There was marked dysdiadochokinesis and bilateral past-pointing. Upper limb reflexes were absent (biceps C5, supinator C6, triceps C7).

M's feet were cold but colour was normal bilaterally and all pedal pulses were present. There was some wasting of the small muscles of the foot bilaterally, bilateral pes cavus deformity and cocking of the toes. Tone was normal in the lower limbs but power was decreased bilaterally (grade 4 out of 5 – slight weakness). There was loss of light touch sensation on the anterior aspect of the tibia (L4, L5) and lateral aspect of the foot (S1) bilaterally. In addition there was loss of proprioception in the distal interphalangeal joint of the first toe bilaterally. Bilateral lower limb ataxia was present and M was unable to perform the heel-shin test on either side, with eyes either open or closed. Deep tendon reflexes were absent (knee L3,L4; ankle S1, S2) and there was an extensor plantar response bilaterally (L5, S1, S2).

M had a pronounced kyphoscoliosis. However, there was normal chest expansion and good air entry bilaterally. Heart sounds one and two were normal, with no added sounds or murmurs present. A subcutaneous insulin pump was in situ in the left periumbilical region.

SUMMARY

M is a 16 year old girl who was admitted electively for a multidisciplinary review of Friedreich's Ataxia, to include investigation of existing cardiomyopathy, kyphoscoliosis and peripheral neuropathy. Over the last two months her left arm has become increasingly weak, with loss of co-ordination. M also suffers from Type 1 DM and hypothyroidism.

M is unable to walk and uses an electric

wheelchair. Findings of note on neurological examination were dysarthria, nystagmus, small-muscle wasting of hands and feet and wasting of proximal left arm muscles. There was weakness, loss of co-ordination and absence of deep tendon reflexes in all four limbs. There were extensor plantar responses bilaterally. Sensation was diminished in the dermatomes of L4, L5 and S1 bilaterally. There was loss of joint proprioception in both lower limbs.

DIFFERENTIAL DIAGNOSIS

The primary differential diagnosis of monoparesis and loss of coordination of the left arm is of mononeuropathy of the ulnar nerve (C7-T1) due to compression. The ulnar nerve is particularly vulnerable to damage at the elbow.[1] Peripheral neuropathy, possibly with a diabetic component, is clinically present in the patient's lower limbs and probably to a lesser extent in the upper limbs. M is left-handed and as existing neuropathy predisposes to further nerve damage, prolonged amounts of time leaning on the left elbow to control her electric wheelchair could cause M's symptoms.

Progression of ataxia due to increased spinocerebellar degeneration should also be considered in the differential diagnosis. Friedreich's Ataxia is a progressive condition, meaning that continued degeneration of M's spinocerebellar function is expected. However, to date loss of power and co-ordination has been largely symmetrical. In this instance the left side alone is affected.

It is possible that M's symptoms are the result of a focal nerve lesion due to diabetic neuropathy. Diabetic neuropathy is most commonly a symmetrical polyneuropathy but focal neuropathy can occur.[2] M is young to have developed clinical diabetic neuropathy but the risk is higher given her background history of neuromuscular degenerative disease and poor diabetic control. However, M's blood glucose control has been much improved for the last twelve months.

In cases of monoparesis, a stroke should form part of the differential. A lacunar infarct in the middle part of the internal capsule's posterior limb can produce pure motor hemiplegia ('ataxic hemiparesis'). A stroke involving the cerebellum could produce co-ordination loss.[3] Cardiomyopathy may predispose to hypoxic or ischaemic damage to the cerebral cortex although damage is usually mild.[4] As the onset of symptoms in this case has been gradual, a stroke is unlikely to be the cause.

PROPOSED MANAGEMENT

Patients with unexplained monoparesis and loss of co-ordination should undergo nerve conduction tests. The symptoms should be observed to identify any deterioration or improvement. M should be advised to reduce time spent leaning on her left elbow and possibly try to control her wheelchair with her right hand for a time to see if this brings about an improvement in her symptoms.

As M has been admitted for review of HOCM, an echocardiogram should be performed and compared with previous reports to identify any progression of cardiomyopathy. An electrocardiogram should be obtained to look for T-wave changes characteristic of HOCM.

Kyphoscoliosis can be assessed by a scan to assess the degree of antero-posterior and lateral curvature of the spine. Pulmonary function tests can be used to determine whether air entry is restricted. An evaluation of overall severity of the deformity and its impact on respiratory function will allow the team to see if it is severe enough to benefit from surgical intervention.

A physiotherapy review should include limb use, activities of daily living, joints, exercises and assessment of the pes cavus deformity.

M's diabetes should also be reviewed. Her blood glucose recordings should be reviewed, as should her HbA_{1C} level. As M is using the subcutaneous method of insulin delivery, the adequacy of doses should be considered and a dietician review obtained. Blood pressure and serum cholesterol levels are simple but important investigations in any diabetic patient. M reports a good foot-care regime but this should be reassessed and appropriate advice offered.

Urinalysis is necessary to rule out asymptomatic urinary tract infection. Psychological support for the patient and family is important and may especially be required given M's poor diabetic control in the past. Thyroid function tests should be performed to assess M's hypothyroidism. A coeliac screen may be appropriate given the existing diagnoses of diabetes mellitus and hypothyroidism.

FRIEDREICH'S ATAXIA

Friedreich's Ataxia (FA) was first described by the German physician Nikolaus Friedreich (1825-1882). He published a series of papers on the condition between 1861 and 1876.[5]

Epidemiology

The prevalence of Friedreich's Ataxia is thought to be one to two per hundred thousand of the population.[2]

Genetics

Friedreich's Ataxia is an autosomal recessive spinocerebellar degenerative disease. This means that once a couple have one affected child, the subsequent risk of another affected child is 25%. The risk of a subsequent child being a carrier (as in this case) is 50%.

The ataxia results from a mutation of the gene on chromosome 9q that encodes for a protein called Frataxin.[6] This results in spinocerebellar degeneration.

Neuropathology

The ataxia is due to a combination of sensory neuropathy and degeneration of both cerebellar afferent neurons and efferent neurons from the dentate nuclei. Peripheral sensory nerves are also severely affected. M's peripheral neuropathy may be complicated by poor diabetic control. Diabetic neuropathy has a multifocal causation; some focal lesions may be of ischaemic origin, while others are due to the susceptibility of the diabetic nerve to compression.[2]

Diagnostic Criteria (Harding's criteria)[7]

A noticeable omission from Harding's criteria (table 1) is HOCM. However, in some more recent studies over sixty percent of patients have been diagnosed with HOCM.[6]

Clinical Features

Onset typically occurs before 20 years of age and usually between 8 and 15 years. Late-onset cases have been described.[8]

Gait Ataxia

Presentation is usually with ataxia of gait. This manifests as increasingly slow and clumsy walking.[8] This is typically followed by limb ataxia, dysarthria, distal loss of joint position and vibration sense, upper limb wasting, generalised areflexia, pyramidal lower limb weakness and extensor plantar responses.[1]

Kyphoscoliosis

Kyphoscoliosis and foot deformities such as pes cavus are common. Kyphosis refers to exaggerated forward curvature of the spine, while scoliosis is lateral bowing. Severe kyphoscoliosis may reduce lung capacity and increase the work involved in breathing.[9]

Hypertrophic Obstructive Cardiomyopathy (HOCM)

Cardiomyopathy occurs in most cases of FA and is accompanied by ECG changes, for example widespread T-wave inversion. This is an important aid to the diagnosis of cardiomyopathy.[4]

HOCM is abnormal hypertrophy of the muscle in the left ventricular or right ventricular outflow tract. In FA an echocardiogram (ECHO) will often show normal systolic and diastolic function, typically with concentric hypertrophy of the myocardium. In Friedreich's Ataxia, HOCM usually runs a more benign course than that of the genetic type and arrhythmias are rare.[4]

Symptoms of HOCM include dyspnoea (as in M's case), angina (this may explain M's episodes of chest pain) and syncope. The arterial pulse may be sharp-rising and jerky and the jugular venous pulse may have a prominent a-wave due to forceful atrial contraction against a non-compliant right ventricle. On palpation there may be a double or triple apical impulse. A late systolic murmur at the left lower sternal border may be present on auscultation and a fourth heart sound (S4) may be present. None of these signs were present in this case.[4]

Abnormalities of coronary arteries can also occur in HOCM.[4] M has not had a coronary angiogram but is at high risk given her poorly controlled Type 1 DM.

Table 1. Harding's Criteria

Essential criteria	**Additional criteria** (present in 2/3 or more)	**Other features** (present in less than 50% of cases)
Onset before 25 years of age	Scoliosis	Nystagmus
Autosomal recessive inheritance	Pyramidal weakness of lower limbs	Optic atrophy
Ataxia of limbs and gait	Absent upper limb reflexes	Deafness
Absent knee and ankle jerks	Loss of vibration and joint position sense in the legs	Distal muscle wasting
Extensor plantar responses	Abnormal ECG	Diabetes
Motor conduction velocity greater than 40m/s	Pes cavus	
Small or absent sensory nerve action potentials	Cardiomyopathy	
Dysarthria within 5 years of onset		

Type 1 Diabetes Mellitus (Type 1 DM)

Ten percent of patients with FA will develop Type 1 DM. In M's case, however, diabetes appears to be a coincidental finding; she presented with diabetes three years before the onset of her first symptoms of Friedreich's Ataxia and a maternal and a paternal aunt, who are not carriers of the abnormal FA gene, also suffer from Type 1 DM.

Life Expectancy

Most patients with FA will die before the end of their 4th decade. Harding et al found the average age of death to be 37.7+/- 14.4 years, with a range of 21 to 69 years.[10] Death occurs from cardiac origin in 5% of cases.[4] In others, respiratory restriction due to kyphoscoliosis ultimately leads to death.

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