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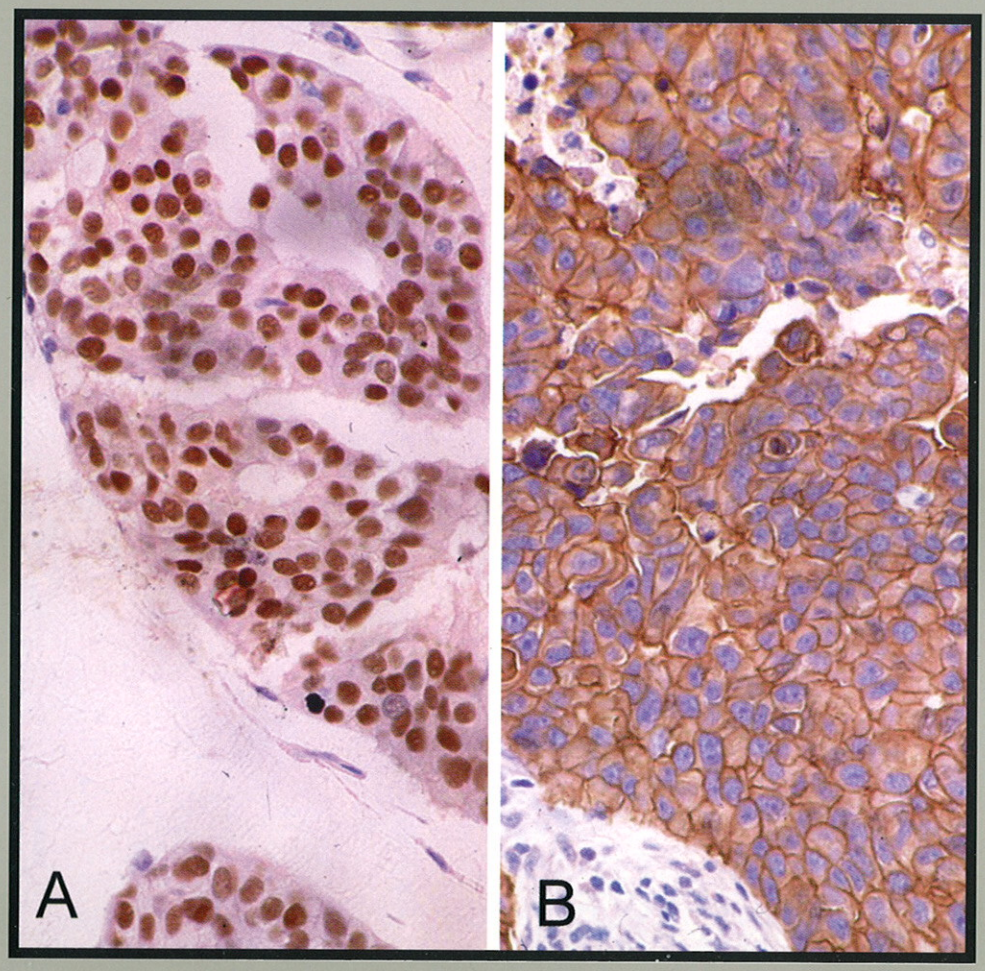

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Cover

Immunoperoxidase staining of breast carcinoma.
A. MAb to estrogen receptor, showing nuclear positively.
B. MAb to oncogene c-erbB2, showing intense membrane and cytoplasmic staining.
(Courtesy of Pathmanathan R, Department of Pathology, Faculty of Medicine, University of Malaya)

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JUMMEC welcomes manuscripts on all aspects of medicine in the form of original articles, case reports, review articles, clinico-pathological conference abstracts and letters to the Editor. Manuscripts should be submitted to:

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Manuscripts: All manuscripts should be submitted in duplicate, typed on one side of A4 size paper and double-spaced with at least 2.5 cm margin. A computer diskette (3.5 in) containing the manuscript in MS Word 6.0 or Word Perfect and a covering letter, stating that the work has not been published or under consideration for publication elsewhere, should be submitted to the Editor. Presentations at meetings do not class as prior publication. The text of the manuscript should be in the following form:

Title page: The title page should contain a concise title of the article. It should identify all the authors, the name(s) of the institution(s) and their full addresses where the work was carried out. The initial and address of the corresponding author should also be indicated.

Abstract and Key Words: The second page should contain an abstract of about 150-200 words. It should state the purpose of the study, a brief description of the procedures employed, main findings and principal conclusions. Three to ten key words should also be listed below the Abstract.

Text: Wherever possible, the text should consist of an introduction, materials and method, results, discussion and references.

References: Number references consecutively in the order in which they are first mentioned in the text. References in the text should be indicated by a figure within parenthesis. The titles of journals in the list should be abbreviated according to the Index Medicus. Authors are responsible for the accuracy of all references. Examples of correct forms of references are given below:

i) ***Journal reference:***
Roberts CW, Alexander J and Bossi L. Studies on a murine model of congenital toxoplasmosis. Parasitol 1992; 104: 19-23.

ii) ***Personal author(s) of book:***
Osler AG. Complement: mechanisms and functions. Englewood Cliffs: Prentice-Hall, 1976.

iii) ***Chapter in book:***
Weinstein L., Swartz MM. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, Eds. Pathologic physiology: mechanisms of disease. Philadelphia: WB Saunders; 1974; 457-472.

Tables: Type each table on a separate sheet and number in arabic numerals. The tables should be as few and as simple as possible, with the title above and any notes or description below. Explain all abbreviations.

Figures: Graphs, drawings and photographs should be submitted as clear, glossy prints measuring 12 cm by 17 cm. Figures should be identified on the back with the title of the article and figure number (in light pencil) and an arrow to indicate the top. Legends to the figures should be submitted on a separate sheet. Explain all abbreviations and symbols used.

EDITORIAL

USE OF ANIMALS IN MEDICAL RESEARCH

The last two decades has seen a fiery but yet unresolved debate on the value of using animals in medical and scientific research, drug testing and education. Proponents for the use of animals in research cite numerous instances why animals are necessary in the investigation of newly emergent diseases, and to elucidate mechanisms and novel therapies for diseases of old.

Historically, animal research has provided answers to many debilitating viral and bacterial diseases that have afflicted man, from clarifying concepts on transmission and pathogenesis of disease to the development of vaccines; fostered the introduction of new drugs (sulfonamides, penicillin); made possible the development of open-heart surgical procedures, organ transplantation and treatment of renal-failure. Extraction from animal tissues of new hormones (insulin) and drugs (heparin) has proven a boon to the therapy of many illnesses.. and the list goes on. Animal activists on the other hand contend that animal research is wasteful and misleading and in turn cite instances when such experiments have failed to address the pertinent health problems of our era. They stress that the uniqueness of animal biology, the unphysiological means whereby disease is introduced in the test subjects and the stresses of the laboratory environment introduce irregularities that are irrelevant to human pathology and therefore that all such testing is a waste of time and money. These individuals go on to cite failed monkey experiments of the early 20s and 30s, which led to misconception on the natural biology of the polio virus, delaying preventive measures, the early development of a vaccine, and finally when vaccine was synthesised from virus cultured in monkey cells, it potentially exposed many humans to potentially harmful monkey viruses. They speak of failed and inconclusive early animal experiments which delayed the implementation of anti-tobacco measures and point out data originating from the U.S. General Accounting Office review which found 52% "serious postapproval risks" in 198 of 209 new drugs marketed between 1976 and 1985, which were not predicted by animal testing.

There are also other ethical considerations. Although there is still much disagreement among scientists in judging pain and suffering in the housing and use of research animals, among the present day challenges are to address and characterise these issues and developing techniques and methodologies that eliminate such suffering. In recent years too, we have come to appreciate that animals have tremendous complexities in life, communicative abilities, social structures and emotional repertoires. The Netherlands in 1996 passed into law that animals have "intrinsic value", are sentient beings and are entitled to the moral concerns of humans.

In the light of all this, most scientists are agreed that some form of cost-benefit analysis should be performed to ascertain if the costs of animal pain, distress and death are counterbalanced with the benefits of acquisition of new knowledge and the development of new medical therapies for humans.

World-wide, the numbers of animals being used in laboratory experiments is declining and in many countries in the West, the total figures have dropped by almost half since the 1970s. The work of animal-rights activists such as that of the Australian philosopher Peter Singer and ethologists Dian Fossey and Jane Goodall have certainly played a role in fuelling the passage of laws regulating animal experimentation. Nevertheless, there is also a definite change in the mentality of modern-day researchers, who growing up in a later era, are alive to these concerns, acknowledge the inherent moral dilemmas of animal experimentation and have also imbibed the concerns of British zoologist William M.S. Russell and microbiologist Rex L. Burch who put forth the "three Rs" in their book "The Principles of Humane Experimental Technique". The 3 Rs exhort animal researchers to : **r**eplace animal by *in vitro* methods wherever possible; **r**educe animal numbers by means of statistical techniques; **r**efine experiments so that animals do not suffer. Although these principles of "**r**eplace, **r**educe and **r**efine" were set forth in 1959, it has taken the better part of twenty years to be accepted in scientific circles.

Present day researchers have at their disposal a vast armamentarium of modern research tools which they should be encouraged to exploit. These include epidemiological studies and clinical intervention trials, careful clinical record-keeping and laboratory testing, *in vitro* testing on human and cell cultures, autopsy examinations, utilisation of microinvasive or non-invasive imaging studies and the use of molecular epidemiological studies. Data emerging from recent research trends in atherosclerosis and HIV research are eye-openers because these emphasise how much new information can be derived while eschewing experimental animal studies. After all, it must never be forgotten that animal "models" are, at best, analogous to human conditions, and no theory can be proved or refuted by analogy alone.

The scientific community is now witnessing changing trends in the sense that humane organizations and government agencies are now investing in and funding research in alternative methods although this is more evident in Europe than in the US. Since 1992, a body has been set up by the European Commission, the Centre for Validation of Alternative Methods, which has an annual operating budget of US $9 million. Statistical sophistry is allowing the classical LD50 test for animal toxicity to eliminated and replaced by protocols which call for reduction of the number of animals used from 200 to 20. The Organization for Economic Cooperation and Development requests that between 3 and 18 animals be used - if the substance being tested kills the first three, no further testing is required. Similar modification in the LD80 for vaccine testing are being proposed, to greatly reduce the number and suffering of animals used. The use of "data mining" techniques has yielded interesting new findings that allows further purification of animal experimentation methodologies. Horst Spielmann of ZEBET, the German centre for alternatives to animal testing, "mined " decades of accumulated data from industrial testing of pesticides and concluded that if mice and rats proves sensitive to a chemical, it does not have to be tested on dogs. Through the activities of ZEBET, production of monoclonal antibodies in tumour-carrying mice has reduced significantly in Europe, as alternative methods are explored. *In vitro* cell lines have supplanted the use of animals in the production of many vaccines and hormones, the most telling success story being that of the production of the polio vaccine. Biomembranes such as Corrositex are now being used in place of the shaved skin of live rabbits for skin corrosivity testing. Cosmetic companies are also reducing animal testing, relying largely on using chemicals tested previously. In medical schools, alternative teaching tools are used, which include the use of multimedia and virtual reality to re-create clinical scenarios; the use of human cadavers to hone surgical training skills is also being actively advocated.

In the US, more than a third of the medical schools do not use animals in their regular curricula. Other changes include the mandatory requirement that all animal experimenters require specific training and licensure before being allowed to do animal related research work. In several medical schools, there is an added emphasis in undergraduate curricula for students to explore and think of experimental methods alternative to animal research.

The Animal care and Use Committee (ACUC) of the Faculty of Medicine was formed in 1988 with a composition of 9 members and empowered with the following terms of reference: "approve the uses made of animal subjects in all animal research studies; to review all animal studies for appropriateness and quality of the animal models; critically evaluate for the humaneness and appropriateness of procedures and conditions surrounding the animal subjects before and throughout the study; evaluate the animal research facility at least annually and to recommend appropriate action to correct deficiencies noted." Although these terms were formulated to reflect the prevailing philosophies on animal care research of the late 80s, it is noteworthy that the Faculty ACUC has endeavoured to promote and practise the same principles which remain current even today.

The ACUC records show that since its inception, approval of animals for experimental use in the Faculty (and in some instances, the University) has gradually increased and seems to be attaining a plateau presently. In 1992, a single request from the Department of Anatomy was given approval by the Committee. By 1996, the number of

projects approved by the ACUC had risen to 25. Aside from the Faculty of Medicine, the Faculty of Dentistry, Department of Genetics and Cell Biology and IPT, as well as government agencies such as PORIM use the Central Animal Facility for research.

It is the present Faculty thinking that at least some kinds of animal research are worth doing as the there is no other alternatives available, and the expected results of such animal studies are conceivably beneficial to humans. But in turn, animal researchers must be painfully aware that since animals may be physically (and perhaps emotionally) such good models for human conditions, then a moral and ethical dilemma exists in using them. We must acknowledge the debt we owe our fellow creatures and support endeavours and measures to achieve the maximum possible gain in scientific knowledge with the least cost in numbers and suffering to the animals.

R. PATHMANATHAN

SPECIAL REVIEW

SCREENING FOR BREAST CANCER

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It is 100 years since William Stewart Halsted described his radical operation for breast cancer by which the entire breast, the underlying pectoral muscles and the lymphatic contents of the axilla were resected in continuity (1). By designing the operation on anatomical principles, Halsted hoped to improve on the unacceptably high local recurrence rates which then followed surgery for breast cancer, many cases of which presented at an advanced stage. Erroneously he also came to believe that 'If three years had passed without detecting either local recurrence or symptoms of internal disease, one could feel sure that cure had been achieved'. By convention the period after treatment at which cure was assumed was extended to 5 years, and later to 10 years, but it is now clear from long term follow-up studies of patients treated only by radical local surgery and radiotherapy that these time-intervals are still too short and that an excess mortality from metastatic breast cancer remains for 30 or 40 years after treatment (*Figure 1*) (2-4). Not that 'personal cures' do not occur; about one quarter of women do not experience detectable metastases during their life-time; but a proportion of these will have died from other causes. Statistical cure of breast cancer, by which a cohort of women with the disease can expect a similar survival rate to that of age-matched women in the normal population has not been demonstrated.

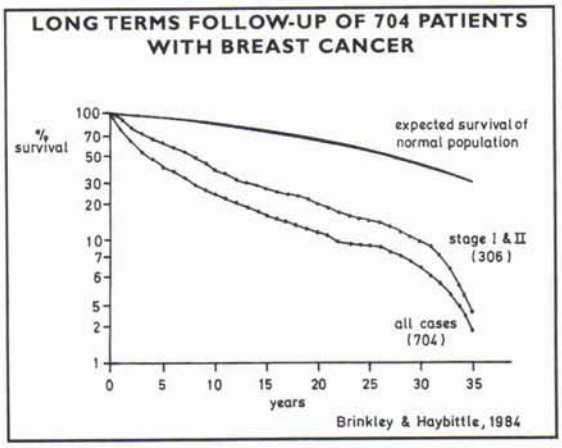

Figure 1: *The classic study of Brinkley and Haybittle which indicated that following the local treatment of breast cancer excess mortality persisted for 35 years (from Brinkley and Haybittle. Lancet, 1984; 1: 1118 with permission).*

Natural History

Halsted subscribed to the view, based on the post-mortem studies of Sampson Handley, that breast cancer spread from its primary site by a process of centrifugal permeation of the lymphatics by a column of cancer cells which advanced to reach the regional lymph nodes (5). There they were believed to remain dormant until such time as the defences of the nodes were breached when 'secondary spread' to bones, viscera and other systemic sites occurred. Embolic spread along lymphatic vessels was regarded as unimportant, such cells being 'filtered' out by the nodes. The potential threat of venous embolisation was disregarded. The natural history of 'early' symptomatic breast cancer was firmly established as that of a loco-regional disease, for which radical removal of the breast and its regional nodes offered the only hope of cure. When it became realised that local recurrent disease also was not prevented, the scope of radical surgery was extended by removing lymph nodes in the neck and from within the chest and postoperative radical radiotherapy was prescribed to destroy residual cancer cells, but, to no avail. The concept that breast cancer was a disease which disseminated late is now no longer tenable.

Breast cancer starts by malignant transformation of the epithelial cells of the terminal ductules within the breast lobules which through a series of genetic changes acquire the properties of unrestrained growth and division (6,7). At this stage the cells do not invade normal tissues but are confined within the basement membrane which maintains the integrity of the epithelial layer. They proliferate only within the lumen of the duct forming an in-situ cancer. The acquisition of invasive properties requires a further series of genetic changes which lead to the expression of proteins by the cancer cell which 'unstick' it from its neighbours, degrade the extracellular matrix and promote migration (8-10). Once this stage is reached the cells penetrate normal tissue barriers, gain access to lymphatic and venous channels, where they are transported to regional lymph nodes and distant sites. In these new sites these processes are reversed. Only a few of the millions of cells liberated from the primary tumour

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withstand the stresses within the vascular system and resist normal host defences, but these will target a capillary in a new site. Having established themselves they migrate through the capillary wall, invade the host tissues, acquire a new blood supply, proliferate a: ' grow under the influence of factors elaborated by them and by normal host cells. At this stage these deposits of surviving cells are but 'micro-metastases' which may or may not survive, or presumably may remain dormant for many years. But potentially they are the forerunners of gross metastatic disease and death. Breast cancer is a chronic progressive disease which disseminates early but recurs late .

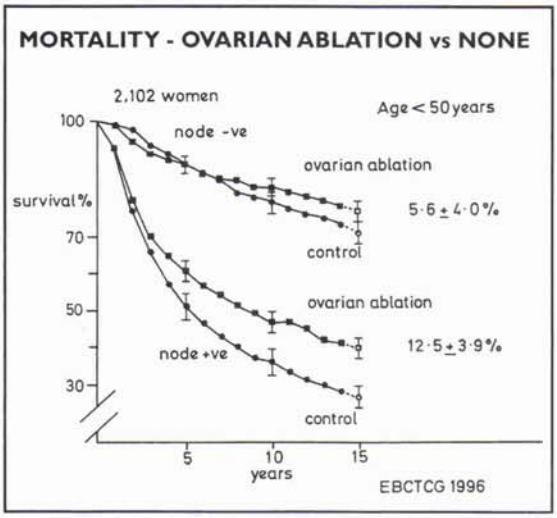

Figure 2: *The outcome of trials of ovarian ablation as adjuvant systematic therapy in early breast cancer in women with node-negative and node-positive disease. (redrawn from Early Breast Cancer Triallists Collaborative Group. Lancet, 1996; 348: 1189-96 with permission).*

Evidence to support the concept that the behaviour of these micrometastases determines the outcome of treatment has come from the effect of systemic treatment by antioestrogens and chemotherapy given as an adjuvant to local therapy. A large overview analysis of worldwide randomised trials which include over 75,000 women has proved that the annual odds of recurrence or death are reduced by 25% following ablation of ovarian function in women less than 50 years of age and by the anti-oestrogen tamoxifen and multiple-agent chemotherapy at all ages *(Figure 2)* (11,12). An absolute reduction in mortality is apparent in women both with involved and non-involved axillary lymph nodes, which at 10 years approximates 10% in node-positive and 5% in node-negative patients. Although this reduction may appear modest, it is equivalent to a reduction or delay of 100,000 deaths for every million women with breast cancer. The recent dramatic improvement in mortality from breast cancer observed in UK is believed largely to be due to the increasing use of systemic therapy as part of initial treatment (13).

A Threshold

An essential principle of screening for any disease is that the test detects the disease at an earlier stage at which treatment confers greater benefit than when delayed until it has become 'symptomatic' (14). For breast cancer this crucial early stage is that before it has disseminated with the formation of micro metastases. Non-invasive cancer is clearly at such a stage, but the potential long time-span of its course may prevent its early detection having an early beneficial effect on mortality. The success of screening for breast cancer depends on whether there is a detectable early stage of invasive cancer before micrometastic disease has been established.

Micrometastatic involvement of the axillary lymph nodes provides clear proof that the disease has disseminated. Its incidence is directly related to the size of the primary tumour. The smaller an invasive breast cancer the less likely is it to have metastasised to the axillary lymph nodes and the better the outcome of local treatment. A recent analysis of 24,740 women with breast cancer included in the Surveillance, Epidemiology and End Results (SEER) programme of the National Cancer Institute has confirmed these relationships (15). For 1,335 women within this series who had tumours less than 1 cm in diameter 5-year survival averaged over 95%. Involvement of the axillary lymph nodes is only one indicator of micro-metastatic disease. Another is the development of clinical evidence of metastases. This was the index used in a French study of 2,648 women in which the size of the tumour measured at the time of primary local treatment was found to be linearly related to the subsequent development of clinical metastatic disease over a follow-up period of 25 years (16). When the diameter of the tumour was 1 cm or less the likelihood of dissemination was less than 20%. Within each size of tumour there was great variation in the likelihood of dissemination, confirming the importance of other biological factors in determining the outcome of treatment. That some of these characteristics may also be time-dependent is suggested by findings that screen-detected invasive cancers may be of less aggressive histological type (17).

A tumour of 1 cm in diameter contains 1 million cells. As with estimated doubling times of between 2-5 months it would take 5-15 years of exponential growth for a cancer to replicate from a single cell, there may be a long period of time during which breast cancer is present in a subclinical phase (8). Contained within this phase is a period (the sojourn

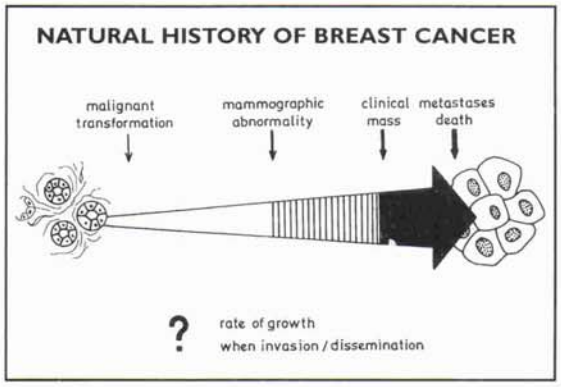

Figure 3: *The natural history of breast cancer to indicate objective of screening (from Forrest. Breast Cancer: the decision to screen. Nuffield Provincial Hospital Trust, 1990 with permission).*

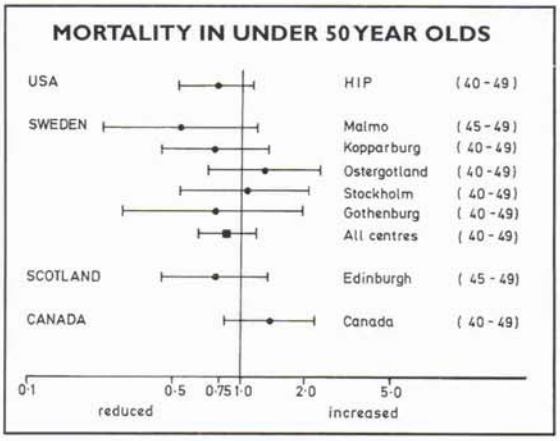

Figure 4: *7-12 years mortality in all randomised trials of population screening by mammography. (from Dixon and Sainsbury. Handbook of Diseases of the Breast. Churchill-Livingstone, 1993 with permission).*

time) when the tumour is detectable by mammography. Detection during this phase is the objective of mammographic screening (*Figure 3*).

Trials Of Mammographic Screening

It has long been recognised that clinically occult breast cancer could be visualised radiologically, but it was only with the technical developments in film-screen mammography during the late 1950s, that mammography was proposed as a potential screening test (18,19). In 1963 the first randomised trial of population screening by mammography was initiated by the Health Insurance Plan in New York, to be followed by the Two-county trial in Sweden and two case-control studies in The Netherlands (20-23). All were reported to show benefit. Six other randomised trials have been conducted in Sweden, Edinburgh (Scotland) and Canada which together with these earlier trials include over half a million women (24-31). Evidence from individual trials, from meta-analyses of reported results and from an independent overview of the four Swedish trials has established that in women of 40-74 years of age, mortality from breast cancer is significantly reduced (32-34) (*Figure 4*). In the six trials with an unscreened control group (excluding the Canadian trial) this reduction averaged 22% *(Table 1)* (32). However it was only significant in women over 50 years of age in whom the mortality reduction was 24% (equivalent to 31% reduction in women who attended for screening). In younger women the mean reduction was an insignificant 15%. Similar mortality reductions have been observed in a large UK comparative trial using geographical controls, in two case-control studies in women invited for screening in Holland and Italy, and in the large Breast Cancer Detection Demonstration Project in USA (35-41). There is also now clear evidence that

Trial	Women aged 40-74 years		Women aged 50-74 years	
	number intended to be screened	relative risk of death	number intended to be screened	relative risk of death
Health insurance plan	31,000	0.71 (0.55-0.91)	15,000	0.69 (0.49-0.97)
Edinburgh	23,000	0.85 (0.65-1.12)	17,000	0.85 (0.63-1.13)
Swedish Trials				
Two counties	77,000	0.78 (0.65-0.93)	57,000	0.72 (0.59-0.88)
Malmo	21,000	0.81 (0.62-1.07)	13,000	0.86 (0.64-1.16)
Stockholm	39,000	0.76 (0.50-1.14)	25,000	0.65 (0.40-1.08)
Gothenberg	21,000	0.81 (0.50-1.29)	11,000	0.91 (0.53-1.55)
[Overview of Swedish trials]		[0.77 (0.67-0.88)]		[0.75(0.65-0.87)]
All trials	212,000	0.78 (0.70-0.87)	138,000	0.76 (0.67-0.87)

Table 1: *Meta analyses of published data on 5-10 years mortality in women over 50 years of age included in randomised trials of population screening by mammography in which control group has no form of intervention. (Wald et al. The Breast, 1993; 2: 209-216 with permission.)*

screen-detected breast cancer is more likely to be non-invasive, or if invasive to be of smaller size with a reduced incidence of lymph-node involvement compared to that presenting in unscreened women (*Figure 5*) (42-44).

UK Screening Programme

In 1987 the UK Government accepted the proposals of a Working Group and initiated a breast cancer screening service as part of the National Health Service (45, 46). At that time the only results were from two randomised trials (Health Insurance Plan, New York,

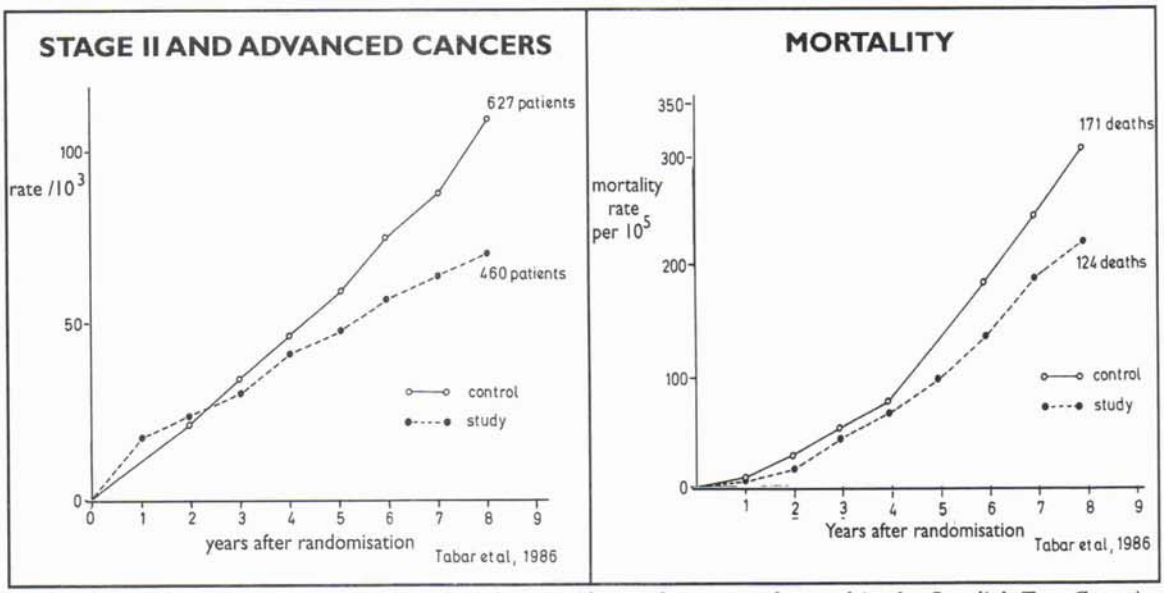

Figure 5: *Cumulative rates of stage II and more advanced cancers observed in the Swedish Two-Counties Trial. (redrawn from Tabar et al. Br J Cancer, 1987; 55: 547-541 with permission).*

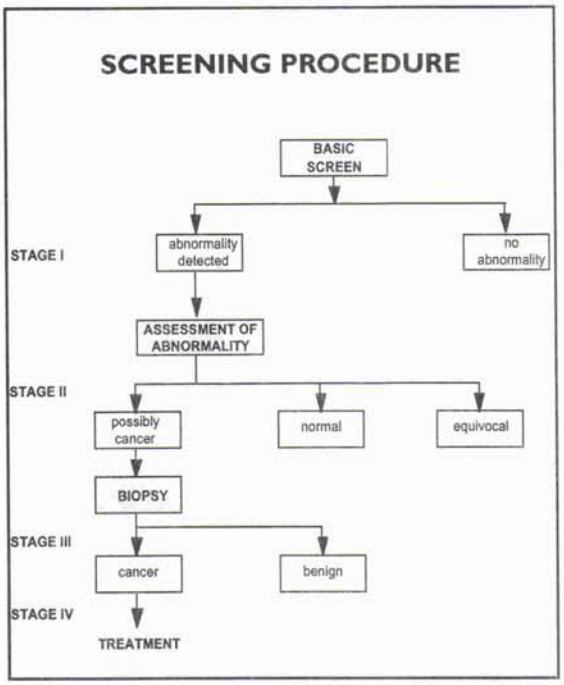

Figure 6: *The Screening Process. (redrawn from Report to Health Ministers of England, Wales, Scotland and Northern Ireland. HMSO, 1987 with permission).*

and Two- Counties, Sweden) and two case-control studies (Nijmegen and Utrecht, Holland) all of which had indicated significant mortality reductions in women over 50 years of age (20-23). In the Swedish two-county trial and Nijmegen case-control study, the screening method used was single medio-lateral oblique mammography; in Sweden, women over 50 years had been invited for repeat screens each 33 months, information which led the Working Group to recommend that the UK programme be restricted to a target group of women of 50-64 years of age who were to be invited for single oblique-view mammography each 3 years. National programmes of mammographic screening were also being set up in Finland, Sweden, Iceland and Norway and since then in Australia, New Zealand, and Canada.

In their Report, the Working Group described four stages in a screening service - the basic mammographic screen, the assessment of mammographic abnormalities, the performance of a biopsy and the treatment of the screen-detected cancer (*Figure 6*). Recognising the problems which might arise were screening to be introduced in an uncontrolled way, they recommended that the screening service should be comprehensive. Steps would be taken not only to provide the neces-

sary mammographic facilities for the 5 million women in the target population, but also the facilities for the evaluation, by diagnostic tests, of those abnormalities detected on the initial screening mammogram, for the biopsy of those lesions suspicious of cancer and for their treatment. This required the institution of a completely new organisation with appropriate management and quality assurance. The UK Government responded by agreeing to support the screening service by 'all necessary back up facilities ... assessment ...diagnostic ... (and) treatment facilities, counselling and after care, and training for key groups of staff' (46).

The Basic Mammographic Screen

It was recommended that the basic mammographic screen should be performed either in urban purpose-built clinics or rural mobile screening vans, equipped with dedicated mammography units. Each unit would perform 12,000 mammograms each year, allowing the screening of 10,000 women in the target population, a small number of older self-referred women and repeat mammograms for those whose initial films were unsatisfactory. With a response rate of 70%, each mammography unit would cater for the needs of 14,000 women in the target population each year, equivalent to a total population of half a million. To cover the whole of UK, 120 units were required. In Scotland, with its population of 5.5 million, 7 static screening clinics were built in the main cities which maintain the servicing of 6 mobile units which travel out into the surrounding country. All films are processed in the main urban units. When it is considered that in Britain there were only 183 diagnostic mammography units in operation in 1985, 128 of which were obsolete, the development of mammographic screening had major implications (45).

Assessment

A basic mammographic screen is not a diagnostic test. It only can separate out those women whose mammogram is normal, is abnormal or of insufficient quality for interpretation. Abnormalities indicating a positive screening test are microcalcifications, discrete opacities (mass lesions), disturbances of architecture and asymmetry. In the British screening service women with abnormalities are recalled to their nearest urban screening clinic which have also been designated as 'assessment centres'; they are not referred either to their general practitioner or directly to the hospital service. These clinics have facilities for clinical examination, sophisticated imaging, fine-needle aspiration and fine needle-aspiration cytology or core needle biopsy which are performed by an expert team including radiologist, clinic doctor, surgeon, histopathologist and cytologist, most of whom also work provide a service to the main urban teaching hospital.

The first step in the investigation of a mammographic abnormality is clinical examination of the breasts, which is facilitated by the examiner (most often a clinic doctor) knowing from the mammogram the location of the suspected abnormality in the breast. If the lesion can be felt, fine- needle aspiration (to determine if cystic or solid) and should it be solid fine needle aspiration cytology are performed leading to a definitive diagnosis in most women. When the lesion is not palpable greater discrimination is required. The tests then used depend on the nature of the lesion.

If a small circumscribed opacity is visible on the mammogram, ultrasonic visualisation will determine whether it is cystic or solid. If cystic, it can readily be aspirated through a needle passed alongside the ultrasonic probe. Should it be solid, magnification mammography is the next step to allow assessment of the border of the lesion. Should this be spiculated an invasive cancer is suspected. Magnification views are also essential for the further definition of micro-calcifications, so that their distribution in relation to ducts and other tissues, can be defined, and also for distortions of architecture, when the 'tenting' of the parenchyma caused by a small invasive cancer may become visible. Additional projections will be required to resolve the reason for asymmetry of the breast parenchyma, which may be due to overlapping densities.

As in palpable breast disease, it is desirable to obtain fine needle aspiration cytology or a core needle biopsy from all suspicious lesions. Radiological localisation using stereotaxic equipment is required to guide the needle to the centre of the lesion. Cytology is particularly helpful in the mass lesion, but of little help for the diagnosis of the cause of calcifications, for which many radiologists now prefer core biopsy. Dedicated units for localisation and core biopsy and even laser treatment of small lesions have now been developed on which the patient is recumbent, but these are expensive and most UK centres rely on simpler 'add-on' equipment used with standard mammography units (47).

As has already been indicated, assessment is best carried out by a dedicated multidisciplinary team. As they all will be present at assessment sessions, decisions can be made without the need for cross-referral between different specialists. Performance can be monitored at regular review sessions which stimulates the development of experience and expertise. The nurse counsellor has become an important member of this team, for women recalled for assessment are anxious and require support.

Biopsy

As a result of the availability of these diagnostic methods and the expertise of the multidisciplinary team, open surgical procedures to obtain tissue for diagno-

sis are now rarely required. This is not the case when assessment is carried out by individual practitioners, when biopsy rates are many times higher. In UK, all biopsies of non-palpable lesions are performed by the 'screening team', with surgeon, radiologist and pathologist working together to ensure that the suspect lesion has been removed and is precisely diagnosed. Pre-operative radiological localisation, usually by the insertion of one or more hooked wires, guides the surgeon to the lesion. Immediate radiology of the specimen is mandatory, and it is now accepted that the radiologist is responsible for confirming that the mammographic lesion has been removed before the surgeon closes the wound.

Treatment

Although the screening service has a responsibility to ensure that cancers detected in the screening programme are treated correctly, treatment is normally carried out in an NHS hospital. The specialist breast surgeon associated with the screening service, although also responsible for the care of symptomatic patients, does not necessarily treat all patients in whom cancer is detected through the screening programme. Those with palpable disease may prefer referral to their local hospital, or occasionally to one in the private sector. However, increasingly women are demanding that the multidisciplinary approach which they have experienced in the screening service should also be available for their treatment and future care. Radiotherapist and medical oncologist then become necessary members of the specialist therapeutic team.

Quality

The Working Group emphasised that the quality of each step in the screening process had to be with the highest quality and recommended that a quality assurance programme be established as part of the screening service. Professional bodies including the Royal Colleges of Radiologists, Pathologists and Surgeons established working groups to develop guidelines for each discipline and laid down the initial standards which should be met in an efficient programme. Particular emphasis was initially placed on its radiological aspects; for example it was recommended that to be competent a screening radiologist should read the films from 6,000 women each year, that recall rates in screened women for further tests should not exceed 10%, that cancer detection rates should not be less than 5 per 1,000 screened women (with 1.5 per 1000 with invasive cancers less than 1 cm in size), and that the benign to malignant ratio on open biopsy should not exceed 3:1 (48). These targets, which were defined in 1992, have since been revised to more stringent levels.

Similar performance objectives have been established for all steps in the screening programme, from the initial definition and invitation of women in the target population to the follow-up of those with screen-detected cancer, and the determination of interval cancer rates (49). Performance is monitored locally by the holding of regular review meetings by screening staff, regionally by the establishment of Quality Assurance Managers with Quality Assurance Reference Centres supported by working groups in each specially, and nationally by the appointment of National Coordinators and coordinating committees in each specially with representatives of each of the 14 health service region of England, and Scotland, Wales and North Ireland. As stated in the 1993 NHS Review of the screening service 'quality assurance is at the heart of the programme' (50). So also is training. Four training centres have been established within the programme.

	number	(%/ratio)
Total screened	1,260,609	
Recalled for assessment	67,475	(5.4%)
FNA cytology	13,466	(1.1%)
Surgical biopsy	6,543	(0.52%)
Cancers detected	6,656	(5.3:1000 screened)
In-situ cancers	1,308	(1.0:1000 screened)
Invasive cancers <15mm	2,655	(2.1:1000 screened

Table 2 *National Health Service review for years 1994-5. Total Screening activity (from NHS Screening Programme Review 1996)*

	target	achieved
Acceptance rate	>70	74.9%
Recall rate	<7	7.2%
Biopsy rate	<1.0	0.69%
Benign biopsy rate	-	0.28%
Cancer detection rate	>5.5/1000	5.9
In-situ cancer	10-20%	19%
Invasive cancers <15mm	>50%	52.8%

Table 3 *Meeting of Targets in National Health Service Programme - Prevalent Screen (from NHS Screening Programme Review 1996)*

The NHS Breast Screening Programme was considered by the National Audit Office in 1992, which in their report, regarded the emphasis on specialisation and rigorous quality assurance as being a great strength (51). In 1995 the House of Commons Health Select Committee reported that 'The NHS Breast Screening Programme is a model service' (52).

Results of Screening

Each screening clinic maintains computerised records which are rigorously checked locally and regionally before being transmitted to the Cancer Screening Evaluation Unit in the Institute of Cancer Research in Surrey, England. Results from the programme are published annually (52-54). Some results for the years 1994-1995 are given in *Tables 2 & 3*.

Not surprisingly, acceptance rates in the invited population (50-64 years) were higher for the second and subsequent (incident) screens (89.2%) that for the first (prevalent) screen (74.9%). So also, as one would expect, were rates of recall for assessment of a mammographic abnormality less (3.4% v 7.2%) as were cancer detection rates (4.3 v 5.9 per 1,000 women screened). For the prevalent round 18.6% of the 6,500 cancers detected were non-invasive and 53% were invasive and less than 15 mm in size. In previous reports 10 mm was taken as the 'threshold' for a minimal invasive cancer. During 1993-1994 24% of cancers were in this category.

It is notable that not only is the surgical biopsy rate low (0.7% for the prevalent and 0.36% for the incident screen) but the ratio of malignant to benign histology on open biopsy is now well above unity. Open biopsies for benign disease (0.28% in the prevalent round) are now uncommon. There are variations between Scotland, Wales, N. Ireland and the 14 regions of England are moderate. For the prevalent screen during 1994-5 uptake rates have varied from 64.3% to 84.7%, recall ratio from 5.4% to 9.7%, biopsy rates from 0.56% to 1.19% and cancer detection rates from 5.0% to 8.4% per 1,000 women screened. Whether these reflect differences in screening practice or in the incidence of the disease is not known.

The target set for the detection of small invasive cancers was greater than 1.5 per 1,000 woman screened, and this would appear to have been met only in 30% of clinics (54). Interval cancer rates have now been reported from two regions of England, and these are higher than those experienced in the Swedish two-county trial, although similar to those in other studies (56-58). There is concern that the sensitivity of mammography and the frequency of screening may be less than desirable, matters which have been subject to research.

Research

The Working Group recognised that their recommendations were preliminary, and emphasised the need for research to determine optimum strategies. Following implementation of the programme, the UK Coordinating Committee for Cancer Research set up a Breast Screening Research Subcommittee which proposed that randomised trials be initiated to determine the best screening test (one or two-view mammography), the optimum frequency of mammography, the effect of screening women under 50 years of age and the management of non-invasive ductal cancer.

Mammographic Technique

The decision to initially recommend single-view mammography as the basic screening test was based on experience in Sweden and The Netherlands. In the event radiologists increasingly asked for a two-view screen (oblique plus cranio-caudal) for the prevalent round, as was standard for symptomatic cases. The results of several retrospective studies and of a UK randomised trial has justified this approach (14,59).

The UK trial was to include 150,000 women attending 9 English screening clinics who were to be allocated randomly to have a single or two-view view mammogram. The design of the trial allowed comparison of one versus two views in both different women and also the same woman, which gave maximum statistical strength. As a result of this design significant findings emerged after an analysis of 40,163 women and the trial was closed.

These findings indicated a clear-cut advantage to two views. There was a significant fall in recall rates (8.16 to 6.97 per 1,000 in different women), and rise in cancer detection rates (5.52 to 6.84 per 1,000 in the same women - an increase of 24%). Two views also reduced the need for further assessment films on recall, and a lower benign biopsy rate despite similar proportions of in-situ and small invasive tumours. Screening by two views cost more than by one view, but the increase was offset by the reduction in cost of further investigations and the higher cancer detection rate. Two-view mammography is now recommended for the prevalent screen in the UK programme.

In the majority of screening clinics the films are still interpreted by a single radiologist, although in Scotland double reading is now routine. Three studies have shown that double reading by two radiologists increases cancer detection rates by from 9 to 15% (for refs see 60). That most recently reported has also shown that it is better to recall on the consensus opinion of two radiologists (if necessary seeking a third opinion) than on acting on a combination of each radiologist's opinion. Compared to single reading, with a recall rate of 6.9% and a cancer detection rate of 7.1 per 1,000, double reading by consensus gave a recall rate of 4.2%, and a cancer detection rate of 8.0. When recall rates were based on the independent opinions of both radiologists the recall rate increased to 9.2% with only a minimal increase in cancer detection (8.1 per 1,000, The lower recall rates associated with consensus

double reading resulted in a savings in overall screening costs.

An alternative to double reading is currently being explored. This employs high resolution digital scanning of mammographic films from which feature detection algorithms for common mammographic abnormalities have been constructed. Prompting systems to alert radiologists to the site of an abnormality are underdeveloped and will shortly be introduced on a trial basis into a number of UK screening clinics (61). The key question to be answered is whether one radiologist, assisted by computer prompting, can reach the performance achieved by double reading by consensus. On-line direct digitisation of mammographic images is also under study (62).

Frequency of Screening

The recommendation for a 3-year interval for screening was based on the Swedish Two- counties trial. Concern with the high interval cancer rates reported during the third year revised Swedish practice, and in that country, as in Holland, 2 years is now accepted as the routine interval for women over 50 years. A UK trial is in progress to compare screening at an interval of 1 year with that of 3 years to which 130,000 women are to be revisited. No results are available.

Age of Target Population

The age at which women are first invited to be screened is causing great controversy (14,63-65). The American Cancer Society, the American College of Radiologists and the American College of Obstetricians and Gynaecologists still advocate that regular mammographic screening should start at age 40 but European national programmes, other than that in Sweden, restrict entry to women of 50 years or more. The National Cancer Institute (NCI), while accepting evidence on the benefit of screening in those over the age of 50 years to be conclusive, have indicated that the findings in women of ages 40-49 who have been included in randomised trials provide insufficient evidence on which to base an informed opinion (*Figure 7*). It advises that younger women wishing to be screened consult their health professionals to decide whether benefit justifies risks and cost. Although it is suggested that in some trials mortality reductions in young women start to emerge 8 years following the introduction of screening, most agree with the NCI view.

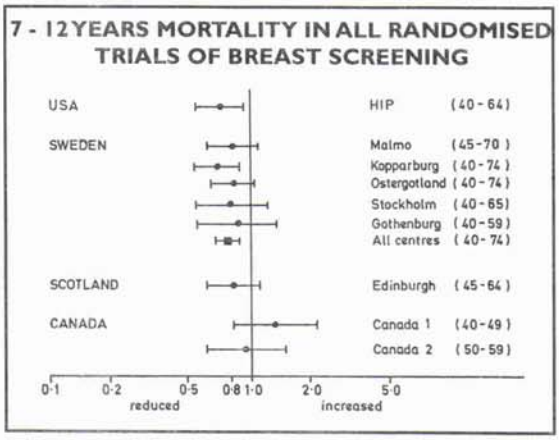

Figure 7: *7-12 year mortality in women of less than 50 years of age in randomised trials of population screening by mammography.*

Information on the effects of screening women less than 50 in randomised trials are based on retrospective subgroup analyses (*Figure 7*). Overviews are confounded also by variations in the method of randomisation, attendance rates, quality and frequency of screening. For a valid answer of the effect of screening younger women, a new generation of trials, such as that now being conducted in UK, are required. In the UK trial 150,000 women aged 40-41 years are being randomised either to be screened annually for 7 years or to await entry to the national programme at age 50. The first analysis of mortality will not be available until 2003, but interim analyses using surrogate indices of benefit, such as tumour size and node status will be performed. In considering the results of screening young women, it is vital that the age at diagnosis of cancer as well as the age at first screen is taken into account. Re-analysis of the overview of Swedish trials using computer modelling has suggested that the greater proportion of the 13% mortality reduction in women of 40-49 years which was observed was due to the diagnosis of a cancer at a later age (63).

The recommendation that the upper age for an invitation to be screened should be 65 years was based on the known reduced uptake in older women. In view of their relatively limited life-span, the benefits which can be expected, in terms of years of life saved, is also less than that in younger women. However, a case-referent study from Nijmegen reported that a mortality reduction from screening persist up to the age of 75 (67). In UK, women over the age of 65 years are encouraged to refer themselves to screening clinics for mammography which is carried out as part of the screening service.

Treatment

Screening programmes uncover large numbers of non-invasive cancers, the significance of which is still uncertain. Non-invasive lobular cancer (lobular carcinoma-in-situ (LCIS)) is regarded only as a marker of increased risk of cancer and only requires regular follow-up mammography. Ductal carcinoma-in-situ

(DCIS) is a frankly malignant lesion which carries a 30% risk of invasive cancer within 10 years, a risk which is greater when it is histologically of comedo type or is of aggressive cytological grade. Some surgeons still advocate mastectomy as the treatment of choice, but in Europe wide local excision is preferred unless the disease is extensive. Only one randomised trial has considered the need for routine radiotherapy following local excision this reporting that recurrence rates following local extension alone were excessive (67). A current UK trial is designed to determine whether the administration of tamoxifen will alter this need. Following local excision of in-situ disease, women are allocated for radical radiotherapy alone, for tamoxifen alone or for both. Over 1,200 women have now been recruited to this trial, the results of which will not become available for several years. It is currently considered that DCIS cannot be considered as a single disease and prognostic factors such as size, the extent of necrosis and cytological grade should be taken into account when planning treatment and must be considered when reporting the results of trials (68).

Invasive breast cancers detected by screening are smaller than those presenting symptomatically, and are more likely to be suitable for treatment with conservation of the breast. The results of several controlled trials suggest that even in small predominantly node negative tumours, radical radiotherapy is a necessary part of conservation treatment (for refs see 71). In a Scottish trial this proved to be the case even when tamoxifen or chemotherapy was routinely given (71). In a randomised trial, conducted by the British Association of Surgical Oncology, patients with small (< 2 cm) and well differentiated (grade 1) cancers are being allocated for radiotherapy and for tamoxifen as in the UK trial of non-invasive ductal cancer.

Prevention

Mammographic screening will not prevent breast cancer. For true prevention the cause of the disease must first be known. This is not the case breast cancer. It has long been recognised that functioning ovaries are a necessary prerequisite to the development of the disease, acting as a reversible promoting factor. Reduction in the period of cyclical ovarian function, for example by an early or artificial menopause, reduces life-time risk, while its prolongation by an early menarche or late menopause increases this. Early and multiple pregnancies are also protective, but whether pregnancy acts through suppression of ovarian function or as a result of differentiation of breast epithetial stem cells is unclear. In the belief that ovarian oestrogen is the hormonal cause of this effect, preventative strategies to reduce the availability of biologically active oestrogens are actively being explored. These include administration of the antioestrogen tamoxifen, reduction in dietary fat and increase in the intake of vegetable products (for refs see 71).

Recognition that maximal proliferative activity of the breast epithelium occurs during the luteal phase of the cycle has led to the suggestion that oestrogen by itself does not stimulate proliferation of breast epithelium, but that progesterone also is required (72). In a young woman the secretion of both oestrogen and progesterone can be abolished by the administration of gonadotrophin releasing analogues, which cause a chemical castration. It is suggested that when the secretion of both hormones are inhibited, it is safe to give small amounts of oestrogen alone to reverse adverse effects. In these circumstances, not only would the incidence of breast and ovarian cancer be reduced, but effective contraception would be provided without the risks of the pill. To prevent an increased risk of endometrial cancer by small intermittent courses of progesterone are given. The feasibility of this approach is under study (72).

The sequencing of the BRCAI and BRCA2 genes with the detection of mutations in those with a dominant family history of breast cancer has raised hope that markers for these genes might allow definition of those also at risk from sporadic disease (73,74). This does not appear to be the case. The carrier state for the mutated genes only defines risk for those with family history of inherited type. In a woman carrying the mutated gene prophylactic surgery can remove risk. But professional counselling on the implications of a family history of breast cancer and genetic testing must first be available to all women.

Breast Cancer In Malaysia

From data collected by the Cancer Registries in Singapore and Penang, it would appear that the incidence of breast cancer in Malaysia is still only about one half of that in the western world. Incidence rates are reported to be lower in Malays than in Indians or Chinese (75,76). At the present time a nationally funded programme of population screening by mammography, such as that in UK, is not likely to be cost effective. As many Malaysians present with the disease at a late stage, public education to increase awareness of the benefits of early diagnosis and treatment should have priority.

However, it is apparent that throughout the cast the incidence of breast cancer is rising and there is a case, as during the 1960-70s in UK, to consider setting up a pilot study of mammographic screening, such as that now being undertaken in Singapore. This

would allow experience to be gained on likely compliance, the sensitivity and specificity of screening mammography in Malaysian women, and also study of the nature of screen-detected cancers. It also could explore the needs for organisation of a population screening service were this later to be desired.

A valuable lesson from the UK NHS screening programme has been widespread realisation of the benefits which a multidisciplinary approach has brought to the management of breast disease. At the time the screening programme was initiated, only a few medical centres had designated breast clinics which practised a multidisciplinary approach the management of symptomatic women. This situation is rapidly changing, and guidelines for the management of symptomatic breast disease within the NHS have been formulated by professional bodies. The availability of such guidelines has great relevance to a country such as Malaysia, which relies to such a great extent on the provision of medical services by practitioners who, working in the private sector, have difficulty in accommodating to a team approach.

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REVIEW

MAGICAL MOMENTS IN MEDICINE
Part 2 - Greek Medicine

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As a prelude towards medicine as a science, the Egyptians acquired a veritable knowledge of diseases and were able to document them on papyri using hieroglyphic writing. Egyptian Medicine influentially reigned supreme for hundreds of years, but the decline came when superstitions and supernatural beliefs started to overshadow common sense and rational thinking. The succulent fruit of Egyptian Medicine that had blossomed from the beautiful flower of civilisation eventually rot, but the seeds of scientific medicine had dropped and got buried in the sands of time, where they would lie dormant for the next 2500 years. It was then that they would germinate and come to flower in the Hellenic Islands of Greece, ushering in the next glorious era of Medicine.

A small coterie of dauntless men - the world's first philosophers - initiated the Greek phenomenon. They dared to *think* and find answers to questions like "What is man and what is Nature?" They thus awakened the human conscience and supposedly bestowed a "time-axis" which is that momentous period in history when philosophers from Greece, prophets from the Middle East, Confucius and Lao-tse in China and Buddha in India created the first great religions and philosophies of mankind, which would form an integral part of Medicine.

The Greeks constructed their infrastructure of Medicine based on two corner stones: *careful observation and rational thinking.* These were sane replacements for blind belief and superstitions which had tainted Egyptian medicine. In the process, two major cults of medicine took form in Greece. The Aesculapian cult or temple medicine, was based on religious counsel and exhortation, coupled with psychotherapy. The empirical medicine, on the other hand, was based on rational thought.

It would be rather inappropriate to talk about the Aesculapian cult without mentioning a word about its founder, Aesculapius, himself. Apollo, the Greek god, in a fit of jealous rage supposedly slew his wife, an earth nymph called Coronis. Her son Aesculapius, however was saved - *in utero* - by Chiron (from which the word chirurgeon is derived), the kindhearted and much accomplished centaur, who later taught him the healing art. Aesculapius, in Homeric days, was a mere mortal, though an excellent physician. After innumerable instances of miraculous healing (including resurrection of the dead), he became an immensely favourite national hero. But his earthly popularity was matched with an equally great measure of heavenly displeasure. He incurred the wrath of the gods, who felt that their prerogatives were under threat. Consequently, Zeus, who considered him as a meddlesome chiropractor, struck him with a thunderbolt so that the gods could retain their power over life and death. Aesculapius was elevated to godhood somewhere around the ninth century.

Among the many children of Aesculapius, Telesphoros, Panacea and Hygeia deserve mention since they dedicated themselves to carry on his good work. Panacea, (which has become an English word synonymous with cure-all or a universal remedy) was believed to possess knowledge of all earth's remedies. Telesphoros symbolized hope and was considered responsible for recovery, while Hygeia (hygiene) tended and fed serpents, which were considered givers of health. Community health was her portfolio.

Picture 1: Aesculapius - The 'mortal' immortal father of Greek Medicine.

The Aesculapian temples were centres of faith healing. They were erected on scenic locations with natural springs and other facilities like stadiums, theatres and bathing pools which are not very much unlike our spas and the modern day health farms situated on hill slopes among lush greenery, complete with a gym, swimming pool etc. Scores of miracle-hungry people made a bee-line to these temples, where they were first screened. The hopeless cases were subtly sent away, which made the cure-rate statistics look impressive. Parturient women were also denied admittance, since birth and death were not acceptable in the premises. The filtered fortunates went through a battery of elaborate procedures, including subterranean baths and foodless diets, before being robed in fine, white linen garments and brought before the mammoth statue of Aesculapius[1]. Possibly drugged by a sneaky dose of a narcotic sedative, the patients drifted off to sleep in the evening, firmly believing that the god would be giving them their healing recipes that night in their dreams. In fact, it is said that dreams were the mainstay of the Aesculapian cult. It is also said that masked priests masqueraded before the drowsy patients in the wee hours of the morning, just to make sure that they "saw" Aesculapius in the flesh. Their cures then came over the air, as if from nowhere, presumably broadcast by ventriloquism. This procedure has not been without slips. One masked (but impatient) "Aesculapius" is reported to have forgotten his act and yelled at his patient: *"Thou art healed. Now pay the fee!"* Nevertheless, most of the patients left next morning, often cured, (or at least thinking so) after making offerings of gold or in kind, according to their means.

The temples also had snakes and other tame reptiles which were trained to lick the ulcerated and swollen wounds of the patients. Enterprising businessmen came out with "snake-biscuits" which were bought by the patients and fed to the snakes in return for the favour. Talking about snakes, (and shuddering even at the thought), I must declare that serpents have had a unique place in History as well as in Medicine. Coming out of the ground, which possesses many healing substances, the snake is supposed to have medicinal powers, including endowment of immortality. Perhaps, after all, Eve was not entirely at fault eating the Forbidden Fruit, considering the fabulous faculties fabled of her accosted friend at the Garden of Eden !

***Picture 2**: The original Aesculapian wand, with a single entwined serpent - the true symbol of the medical profession.*

***Picture 3**: Caduceus, the winged wand of Hermes with a pair of serpents. Erroneously considered synonymous with the Aesculapian wand.*

The staff of Aesculapius, around which a huge serpent lies entwined, has become representative of the healing art and is considered the true symbol of the medical profession[2]. However, this has been frequently and erroneously confused with Caduceus, the magical wand used by Hermes to open the doors between the gods and men. This wand is with wings and usually shows a pair of entwined serpents[3].

The empirical medicine, on the other hand was more rational. It considered disease as a dysfunction of the body and used diet, herbs and drugs in treatment. Diagnosis formed an important part. Nomadic physicians called *periodeutai* and military surgeons practiced this form of medicine. They were said to be proficient in the art of *pronoia* (? pronounce) whereby they rattled off detailed descriptions of the illnesses of patients even before their clients themselves could utter a word, thereby impressing many a gathering at the town square.

One man who practised this form of medicine and who would later go down in History as the Father of Medicine was Hippocrates[5]. Little is known of his personal life. He was born on the Greek island of Cos in c. 460 B.C. and died in Larissa c. 377 B.C. His father was a medical practitioner and he apparently grew up in a medical atmosphere. But it is rather amazing to observe that this great man, like Homer, Christ and Socrates, never wrote a word ! In fact, the *Corpus Hippocraticum*, which contains seventy two volumes, was compiled almost a century after his death by Egyptian scholars. The scholars themselves were commissioned by a book-loving Pharoah, Ptolomy Soter (323-285 B.C) who wanted to bring out a complete, authentic edition of Hippocrates' teachings. It is believed that these scholars in their zealous over-enthusiasm, collected every scrap of writing which had a Hippocratic stamp on it and ascribed it to the mentor. Only less than a third of the treatise is definitely credited to Hippocrates. Whether or not he inspired the rest is anybody's guess. But the book definitely gives us an idea of the advanced state of medical knowledge in ancient Greece, Hippocrates' perspective of disease as a natural process which developed in logical steps and most important of them all, the concept of treating the patient as an individual, whose constitution would react to disease in its own way.

According to Plato, Hippocrates had several students attached to him (for a fee, of course) who followed him in his rounds and learnt the art of diagnosis, prognosis and treatment. After this training, they became free to practice medicine which had much competition, with doctors even resorting to unethical behaviour to woo their patients. Hippocrates had some caustic castigatory advice for them.

Hippocrates, however, was human and bound to err. One grave mistake that he made was to surmise that the body consisted of four humors namely blood, phlegm (brain), yellow bile (liver) and black bile (spleen). It is thought that he came to this conclusion, perhaps, after observing the four layers formed by clotting blood. It took a long time for physicians to get disillusioned of this theory.

While Hammurabi legislated medical codes by law, Hippocrates proposed a moral code for doctors. The Hippocratic Oath[6], (which in present times has frequently become a *hypocritic* one !), is considered the laurel wreath and the crowning glory of his work and in essence states that a good physician must first be a good and kind person. But again, controversy exists as to the authorship attributed. Textual analysts believe that it was the family code of conduct of a medical society called the Asclepiads, to which Hippocrates belonged. In those days, knowledge transmission was from father to son, which is supposed to be the reason for one of the clauses, which states that the student must support his teacher. But whatever it was, it is certain that Hippocrates' reputation as a doctor, together with the noble provisos laid down in the oath, made it an exalted charter of therapeutic code and ethics, accepted by the entire medical profession worldwide.

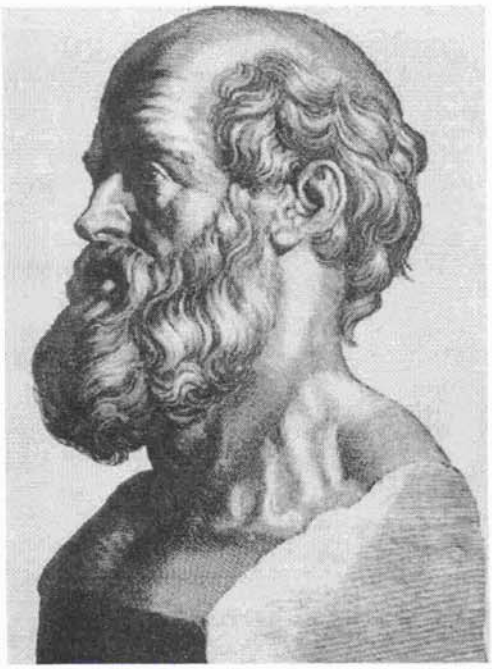
Most of us know Aristotle as a philosopher, but Hippocrates apart, he was the man to make a powerful impact on medicine. He served to organise the facts learnt by the Greeks into a system with a logical infrastructure, prompting Charles Darwin to call him the "world's greatest natural scientist". But unfortunately, many of his speculations and conclusions were inaccurate. He decided - without even dissecting the human body - that the heart was the body's nerve centre and the brain was a bloodless mass of earth and water, whose sole purpose was to regulate the heart. Observing maggots in dunghills and moulds formed on hardening dew, he concocted the theory of "spontaneous generation" whereby creatures were spontaneously brought forth. The fact that King Alexander the Great of Macedonia was his student made things worse, since these inaccurate theories were propagated across the globe through the influence of his world-conquering pupil and perpetuated right till the 19th century when Pasteur called his bluff.

There were several others who also made significant contributions towards medicine but are less known. Heraclitus of Ephesus, who lived in the 3rd century B.C. is considered to be the first true anatomist. He discovered the prostate and named the duodenum (from duo - deka - dactylos or 12 fingers which is the length of the duodenum). He is also acclaimed to have made the distinction between blood vessels and nerves and sensory from motor nerves. After carrying out the first methodical investigation of the brain, he is said to have declared that dreams were a retreat into a personal world and not a journey into supernatural spheres. Alcmaeon of Croton, a student of Pythagoras, laid down the foundations for veterinary medicine by performing animal dissections and was the first to make a distinction of arteries and veins from blood vessels. Parmenides of Elea declared that heat loss was the cause of death. Diogenes of Apollonia pursued a career in comparative anatomy. The pump had just been invented and Erasistratus was quick to surmise that the heart was a modified pump. He is credited with the discovery of the tricuspid valve.

The world's first immunologist was a product of Greece. Mithridates Eupator[4], the paranoid King of Pontus, who lived in ceaseless fear of death from his adversaries, tested poisonous substances on criminals and then downed some of it himself, in ever increasing quantities. He, therefore, became immune against them and unwittingly paved the way for the science of immunology.

Picture 4: *Mithridates Eupator, King of Pontus (132-63 B.C), possibly the world's first immunologist.*

Medical proficience brought a lot of prosperity to ancient Greece. Cyrene island exported bales of *silphos*, which was a spice as well as a medicine. Theriaca, was a exalted drug named after a snake-bite epic written by Nicander, a popular Greek doctor. This wonder drug, according to its manufacturers, was an universal antidote for just about anything. It is said that when Claude Bernard, the famous 19th century physiologist, was a drugstore apprentice, the pharmacist let him into a little secret about Theriac. Thereafter, dumping all the leftovers and unsalable drugs into a big jar and mixing them up thoroughly to create the wonder drug was no sweat



Picture 5: Hippocrates, the Greek physician. (460 B.C. to 377 B.C.)

The Hippocratic Oath

I swear by Apollo the Physician, by Asclepius, by Hygenia, by Heal-all and by all the gods and goddesses, making them witnesses, that I will carry out, according to my ability and judgment, this oath and this indenture. To regard my teacher in this art as equal to my parents; to make him partner in my livelihood and when he is in need of money to share mine with him; to consider his offspring equal to my brothers; to teach them this art, if they require to learn it, without fee or indenture; and to impart precept, oral instruction and all the other learning to my sons, to the sons of my teacher and to pupils who have signed the indenture and sworn obedience to the Physician's Law, but to none other. I will never use it to injure or wrong them. I will not give poison to anyone though asked to do so, nor will I suggest such a plan. Similarly, I will not give a pessary to a woman to cause abortion. But in purity and in holiness, I will guard my life and my art. I will not use the knife on sufferers from stone, but I will give place to such as are craftsmen therein. Into whatsoever houses I enter, I will do so to help the sick, keeping myself free from all intentional wrong-doing and harm, especially from fornication with woman or man, bond or free. Whosoever in the course of practice I see or hear (or even outside the practice in social intercourse) that ought never to be published abroad, I will not divulge, but consider such things to be holy secrets. Now if I keep this oath and break it not, may I enjoy honour, in my life and art, among all men for all time; but if I transgress and forswear myself, may the opposite befall me.

Picture 6: The Hipprocratic Oath, whose authorship is doubtful, but allegedly ascribed to Hippocrates.

and the farmers were supposed to have gladly gobbled up the potpourri, often getting irate when their stocks ran out.

The Greeks recognised the need of the social responsibilities of the doctor and created the post of a city medical officer for public health and advice during epidemics. Some of the towns established public *"jatreia"* which was something like the modern polyclinic. In some places, a health tax was levied to maintain government dispensaries. Medical officers were frequently also assemblymen with political responsibilities and the records state that they discharged their dual duties quite satisfactorily.

Ancient Greece also housed the earliest scientific schools. Archimedes, Euclid, Herophilus of Chalcedon, Father of Anatomy and Erasistratus were all teachers in the Museum (meaning home of the muses) founded in Alexandria in 331 B.C. The place was reputed to have had one of the best libraries in the world, with a collection of well over half a million volumes, but unfortunately was burnt down in an ethnic uprising in 295 A.D. The world's first clinical school and university, were also established here and had laboratories and a cafeteria. Organised anatomical dissections were first practised here which brought in a new concept that the seat of disease was the organs of the body and not the humors as postulated by Hippocrates.

Greek medicine remains, till today, one of the most significant landmarks of western civilisation and the ancient Greeks will always have a noteworthy spot in the annals of Medicine. They assimilated the medical knowledge of the Egyptians, Babylonians and all the neighbouring countries but were sensible enough to rationally analyse the information, discarding elements like magic. Although mythological beliefs were part of the system, their approach was sensible, naturalistic and had some scientific basis. The eventual outcome was objective observation of the patient, revised concepts of diseases and the evolution of a *humane* human physician who understood not only the disease but also the patient as an individual on a mortal scale and his own commitment and mission on earth on a moral scale.

In our epic odyssey on the historical road of medicine, we have just about reached the half-way mark, even in this, the second episode. That is because Hippocrates is separated from Imhotep of Egypt by a time span of over two thousand years, which is roughly the same duration separating Hippocrates and Pasteur. The next half of the journey would be unraveling the events of the past two millenia and so keep those seat belts on and your eyes peeled, while ruminating on these Hippocratic aphorisms:

" Life is short, the art long, the occasion fleeting, experience fallacious and judgment difficult. "

" You must not only do the proper thing, but do it at the right time. "

" Whoever is to acquire a competent knowledge of medicine ought to have the following advantages : a natural disposition; instruction; a favourable position for study; early tuition; love of labour; leisure. First of all a natural talent is required, for when Nature opposes, everything else is in vain; but when Nature leads the way to what is most excellent, instruction in the art takes place, which the student must appropriate to himself by reflection, early becoming a pupil in a place well adapted for instruction. He must also bring to the task a love of labour and perseverance, so that the instruction, taking root, may bring forth proper and abundant fruits... Physicians are many in title, but very few in reality..."

Next : Roman Medicine

REVIEW

NASOPHARYNGEAL CARCINOMA: EPIDEMIOLOGICAL REVIEW IN RELATION TO ITS ETIOLOGY

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Introduction

Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma is a malignant tumor arising from the epithelial cells(1) lining a recess along the lateral wall of the nasopharynx named "Fossa of Rosenmuller" (2,3). It is one of the most rapidly developing tumors of the head and neck and comprises more than two thirds of all tumors arising in the nasopharynx (4). Unlike other head and neck cancers, the natural history of nasopharyngeal carcinoma unfolds certain features, which make this cancer a special entity by itself. One of the most unique features is its geographic distribution. While its incidence among the people living in the Southeastern region of China and Hong Kong and their descendants living in other parts of the world is as high as 30 per 100,000 per year for males(5), its incidence among those living in the rest of the world is less than 1 per 100,000 per year. Besides ethnic Chinese, other local ethnic Mongoloid group of people living in the South-East Asian countries have a relatively higher risk as compared to Caucasians living in the same geographic regions (6). People at intermediate risk to the development of nasopharyngeal carcinoma are those from certain northern African countries (4) like Morocco, Tunisia and Algeria, and from Arctic regions like Alaska and Greenland (7). Another unique feature of nasopharyngeal carcinoma is its incidence at relatively early age. For most cancers, the incidence is low before the age of 55 years which steadily increases after this age with no tendency to decline. For nasopharyngeal carcinoma there is significant rise in incidence (10 per 100,000 per year) from the age of 30 and after reaching a plateau between 40 and 49 years (8) the incidence rate steadily decreases. For the Malaysian Chinese male in the age group 40-49 years, the age-adjusted incidence rate was noted to be as high as 40.1 per 100,000 per year (8). Almost 75% of patients belonged to the age group 30-60 years. This observation was more obvious among patients of Chinese and South-East Asian origin.

So far as sex incidence is concerned the average male to female ratio is 2.5 to 1. This sex incidence has been noted to be almost uniform among the patient population all over the world, without much variation.

It is generally considered that the etiology of nasopharyngeal carcinoma is multifactorial with at least three factors playing their respective roles. The factors are genetic, Epstein-Barr Virus (EBV), and other environmental agent/s which has/have EBV-activating and/or co-carcinogenic function. While considering these factors from the epidemiological point of view it is essential to have the background knowledge of the histogenesis of this cancer so as to interpret the relevance of epidemiological information in relation to its actual development.

The epithelial origin of nasopharyngeal carcinoma is now well established (1,4), and so is the fact that irrespective of the type of cell lining (whether stratified squamous or pseudostratified columnar), it is the squamous cells either original or metaplastic (pseudostratified columnar cells after having undergone metaplastic/dysplastic change) which undergo neoplastic transformation (9,10,11). It has been noted that the basal cells of the pharyngeal epithelia are endowed with receptors for EBV(12) which facilitate its entry. It is felt that the subsequent interaction of EBV DNA with the DNA of basal cells could be the crucial event in bringing about the neoplastic change (13). A question often raised is whether EBV infects normal epithelial cell in the nasopharynx or after dysplastic/metaplastic changes have occured (11). None the less, the presence of EBV DNA has been consistently noted in each and every viable nasopharyngeal carcinoma cell obtained from all parts of world (14). Besides, all the three histopathological types of nasopharyngeal carcinoma (WHO I or the keratinising squamous cell carcinoma, WHO II or the non-keratinising carcinoma and the WHO III or the undifferentialed carcinoma), as defined by the World Heath Organisation (15) have been noted to be associated with EBV (16).

In so far as pathogenesis is concerned there is a need to put together the role of genetics, EBV and other environmental factors which effect the ultimate change (17). EBV, which in normal circumstances remains dormant due to factors yet to be firmly established springs into activity and paves the way for changes in multiple step fashion towards malignancy. Genetic factors inherent in the cells play a role, most probably in all the steps involved.

Epidemiology

Epidemiology can be descriptive (or observational) or analytic. The former states the facts as observed which forms the basis for generating a hypothesis, while the latter attempts to test the hypothesis through laboratory assays using various techniques. These techniques could be used to study various factors like etiology, pathogenesis, diagnosis and control of a particular disease.

In this article an attempt is made to look into the etiological factors involved in the development of nasopharyngeal carcinoma using descriptive as well as analytic epidemiological methods.

Etiological factors

Genetics

With regards to the role of genetics in the etiology of nasopharyngeal carcinoma, the main support is from a descriptive studies which include unique geographic distribution, familial clustering in low-risk population (18) and the increased risk of developing this cancer in individuals with a family history of the disease (19). However the observation of a lower risk among second and third generation migrant Chinese as compared to native Chinese (20) favors a role of environmental factors over genetic factors.

Analytic studies have largely been centered around the examination of specific genetic markers of susceptibility like Human Leucocyte Antigen (HLA) studies (21,22), study of the immunoglobulin allotypes (23), red cell enzymes and serum proteins (24). Association of Chinese nasopharyngeal carcinoma with HLA-A2/BW46 has been observed in several studies (21,25,26) and association of HLA antigens other than A2/BW46 has been observed in non-Chinese population (22,27). In another study (28) involving 30 families with at least two siblings affected with nasopharyngeal carcinoma, a 21-fold increased risk was attributed to a gene closely linked to the HLA locus, still undetermined.

Results of studies to investigate the relation of immunoglobulin allotypes, red cell enzymes, serum protein and other potential genetic markers have so far remained conflicting and not much progress has been made in this direction.

Epstein-Barr Virus

It is generally believed that EBV is etiologically associated with the development of nasopharyngeal carcinoma. Evidences in support of this belief are both observational as well as analytic.

Since the time Old *et al* (29) noted unusually strong precipitating bands against EBV antigens in the sera samples from nasopharyngeal carcinoma patients and Henle *et al* (30) established that immunoglobulin A antibody titer against the viral capsid antigen of EBV was significantly elevated in 93% of nasopharyngeal carcinoma patients, there have been many similar reports in the literature. Furthermore, this titer was noted to have been raised even up to 41 months prior to the confirmation of nasopharyngeal carcinoma in a group of patients observed during a survey conducted in China (31). These sero-epidemiological findings suggest that the change in the state of EBV from dormant to active takes place long before the actual neoplastic change. This increased activity over a prolonged period of time might be a significant contributing factor in bringing about change in the epithelial cells harbouring this virus.

Laboratory evidences in support of an etiological role of EBV in nasopharyngeal carcinoma are increasing rather rapidly. It is established that EBV has carcinogenic potential (32) and that vaccination against EBV provides full protection against tumour development in animals (33). Several EBV antigens like EBV-nuclear antigen 1, EBERs, latent membrane proteins 1 and 2 and Bam HI-A fragment have been noted to be expressed in almost all nasopharyngeal carcinoma (16,24). It has been suggested (3,4) that expression of latent membrane proteins may have a role in preventing the process of apoptosis (programmed cell death), thus leading the cells to immortality. The full significance of all these expressions have remained unexplored so far.

Based on molecular studies (34) of the pre-invasive lesions (dysplasia and carcinoma in situ), related to nasopharyngeal carcinoma, it has been possible to ascertain that these lesions arose from a single EBV-infected cell, which is indeed an early event in the pathogenesis of nasopharyngeal carcinoma. This identification of premalignant clones of EBV-infected cells has been considered (35) to be a remarkable new evidence suggesting that EBV is a primary etiologic agent in the multistep process that leads to the development of nasopharyngeal carcinoma.

Other environmental factors.

Numerous epidemiological studies have been conducted in order to explore the possibility of participation of other environmental factor/s which could play an etiologic role in nasopharyngeal carcinoma. Factors which have been examined in depth include diet, smoke (cigarette, tobacco, incense, anti-mosquito coils), wood, wood dust, formaldehyde, chlorophenols, herbal medicines, nasal oils, alcohol and micronutrients. Results of most of the studies are rather conflicting but those concerning dietary factors seem to have some relevance. Among the studies including dietary factors, the one which has been most extensively studied

involved consumption of salted fish. Ho (37) was the first to propose its involvement in the etiology of nasopharyngeal carcinoma having observed in an epidemiological study that the seafaring Tanka Chinese, a subgroup of Cantonese, who consumed salted fish quite frequently had a two times higher risk to the development of nasopharyngeal carcinoma as compared to land-dwelling Cantonese. Subsequently there have been many studies which support the strong association between consumption of salted fish and the risk of development of nasopharyngeal carcinoma (38,39,40). The risk is more if the salted fish is consumed over several years, since childhood or quite frequently (e.g. daily or weekly).

Besides observational evidence, analytic findings through laboratory studies also support an etiological association of nasopharyngeal carcinoma with the consumption of salted fish. Rats fed salted fish tend to develop nasal cavity tumours (41) and urine collected from them has mutagenic capabilities (42).

Consumption by Chinese of preserved and processed food like salted shrimp paste (39), salted duck egg (43), salted mustard green (43), salted soy beans (44), salted Chinese tuber (44), canned pickled vegetable (44), "Sze chuan chye" and "Kiam chye" (44) have also been incriminated. Certain food taken by Tunisians like harissa, quaddid and stewing mixture have been found to impart an increased risk of nasopharyngeal carcinoma among consumers as compared to non-consumers (45). Laboratory evidence of nitrosamines or their precursors in salted fish and several other foods items have been found (46). Salted fish as well as several of these food have also the capability to induce EBV *in vitro* (44). Yet definitive evidence of a role of the dietary factor or factors in the etiology of nasopharyngeal carcinoma remains elusive.

Discussion

Descriptive as well as analytic epidemiological evidences strongly support a causative role of EBV in the development of nasopharyngeal carcinoma. EBV, a ubiquitous virus, which infects nasopharyngeal epithelial cells normally remains latent and needs to be induced to be active. Suggestions that salted fish and some of other food items have the capability of inducing EBV *in vitro* and that they are endowed with chemical carcinogenic potential, make them favourable candidates for consideration in the list of etiological factors. At this stage one can only speculate about the site and the mechanism of their actions, acting independently or in association with EBV.

That a genetic factor plays a significant role cannot be ruled out. While overwhelmingly large data on geographic and ethnic distributions strongly favor the genetic contribution, there is no substantive analytic support forthcoming so far. Even studies of the migrant status of vulnerable population (20,47,48) seem to dilute the genetic theory. Results of studies of the incidence of nasopharyngeal carcinoma among migrant populations as compared to native ones, have clearly shown that there is a consistent decrease in the incidence among the second generation population as compared to the first generation or the homeland population. These observations favor an environmental role more than genetic. However, in one such study (49) involving Chinese males in Los Angeles, it was noted that the annual incidence rate between 1972 and 1974 was lower (3.5/100,000) for US-born Chinese as compared to foreign-born Chinese (13.9/100,000), but it was pretty high as compared to Caucasians (0.6/100,000). This reflected a residual increased incidence among the second and third generation Chinese migrants who were in US as compared to Caucasians, thus providing support to the genetic theory.

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MINI REVIEW

PERCUTANEOUS ENDOSCOPIC GASTROSTOMY

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ABSTRACT: Since the introduction of percutaneous endoscopic gastrostomy (PEG) into clinical practice in 1980, it has emerged as the preferred method of providing long-term enteral nutrition. PEG insertion is a relatively easy and rapid endoscopic procedure, well tolerated and affords significant palliation to patients. It has been shown to be safe and effective, the rate of complication and mortality being acceptably low. However, PEG is not as widely known or accepted here as it is elsewhere. Patients continue to have nasogastric tubes in place for lengthy periods adding to their discomfort and debility. ***(JUMMEC 1996 1(2): 29-32)***

KEY WORDS: Percutaneous endoscopic gastrostomy, Gastrostomy tube, Enteral nutrition

Introduction

The importance of enteral feeding in providing nutritional support in man has long been recognised from centuries ago with the use of nutrient enemas. With time, the delivery system became more sophisticated with the advent of flexible nasogastric feeding tubes. In the mid-nineteenth century, the surgical creation of a fistula between the stomach and abdominal wall was first attempted and over the next hundred years, feeding gastrostomy involving a laparotomy has evolved to become a well established alternative in providing enteral nutrition (1). The most recent innovation in technology was the introduction of percutaneous endoscopic gastrostomy (PEG) into clinical practice in 1980 (2). This has gained wide acceptance and has become the preferred procedure for achieving prolonged enteral nutrition in patients with the inability to swallow who have a functionally intact gut (3,4,5). PEG placement precludes the need for general anaesthesia and an open procedure. It is adequately performed with local and intravenous sedation. The cost benefits of PEG outweighs that of surgical gastrostomy in terms of being significantly cheaper, quicker to perform and involving a shorter hospital stay (6).

Published papers suggest that long term feeding by PEG is safe, effective, with a low complication rate and low mortality. Both short and long term comparisons of PEG and nasogastric tube (NGT) feeding suggest that PEG is at least as effective if not superior to NGT feeding (4,5). In the comparison of the two methods, Park et. al (4) demonstrated better nutritional status with PEG explained by the fact that accidental removal of nasogastric tubes resulted in patients receiving significantly less of their prescribed feeds than with PEG. PEG seemed better tolerated and cosmetically more acceptable than the NGT. However, there are conflicting results with regards to the rate of reflux and aspiration pneumonia occurring in PEG and NGT feeding (7,8). In order to minimise the risk of gastro-oesophageal reflux, one study suggested that continuous as opposed to bolus gastrotomy feeding may be of help (9). This is demonstrated by the occurrence of scintigraphic reflux and significantly reduced lower oesophageal sphincter pressure on bolus feeding and not on continuous infusion feeding. This suggests that PEG-reflux may be a function of lower oesophageal sphincter alterations caused by gastric distension.

Indications

Patients suitable for PEG are mostly those who have a permanent neurological disorder causing dysphagia such as cerebrovascular accident, head injury with hypoxic encephalopathy, motor neurone disease and dementia or head and neck cancer (3,10,11). In addition, there have been a number of studies focusing on PEG placement in specialised circumstances. A series on burn patients showed PEG to be effective for nutrition and decompression without increasing the risk of wound infection or major complications (12). In paediatric settings, PEG has been used with effect to provide supplemental nocturnal feedings in patients with malabsorption and inflammatory bowel disease (13). The use of PEG in malignant bowel obstruction for gastric decompression has also been reported with a favourable outcome (14,15,16).

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Method of Placement

There are two techniques of inserting the PEG tube: the "push" and the "pull" technique. At the Endoscopy unit, University Hospital, we favour the "pull" technique of Ponsky and Gauderer (2,17). Prior to the procedure, mouth toilet is carried out and prophylactic parenteral antibiotics administered. Topical laryngeal anaesthesia with lignocaine spray is given and intravenous sedation achieved with 2.5 to 5 mg of Midazolam. A thorough endoscopic examination is then performed to visualise the oesophagus, stomach and duodenum to exclude significant pathology which would preclude PEG insertion. The patient is turned from the left lateral to the supine position and the abdomen is cleaned and draped. Following this, the stomach is insufflated with air to create gastric distension which mobilises the liver, spleen and colon away from the gastrostomy site and brings the anterior stomach wall in contact with the abdominal wall. The anterior abdominal wall is then transilluminated with the endoscope. The point of transillumination and therefore, the gastrostomy site is confirmed when finger compression at this spot is appreciated as a localised depression on endoscopic view. 1% lignocaine is used to infiltrate the skin and fascia at this point and a 1 cm skin incision is made with a scalpel. A large-bore needle cannula with inner stylet is then introduced with a quick motion to pierce the abdominal and gastric wall without pushing the stomach away. Endoscopic visualisation ensures proper positioning of the cannula before the inner s tylet is withdrawn. In the meantime, an endoscopic snare is looped around the cannula. A metal loop wire is threaded through the cannula and is grasped with the snare as it exits from the cannula. With the wire firmly secured in the snare, it is brought out through the mouth together with the endoscope.

The gastrostomy tube has a metal loop attached at the tapered end. This is interlooped with the metal loop wire and by pulling at the abdominal end of the wire, the gastrostomy tube is drawn into the mouth down the oesophagus and stomach until the gastrostomy bumper is in apposition with the gastric wall. A repeat endoscopy confirms the proper positioning of the bumper. The tubing is cut to the desired length and an outer bolster is placed to anchor the tube to the abdomen. Finally, a feeding adaptor is screwed onto the gastrostomy tube. The 'push' method is very similar to the 'pull' method but uses a long, firm, tapered gastrostomy tube which is pushed over the mouth end of the guidewire (18,19).

The success rate for either methods of PEG insertion is close to 100%. There are more publications on the experience with the 'pull' than the 'push' method. However, there is little to choose between the two methods. Both have the potential of introducing oral flora into the stomal site and both require two passes of the endoscope to ensure proper positioning of the gastrostomy tube bumper.

A third method described by Russell uses a Foley catheter that is inserted percutaneously directly into the stomach by way of a peel-away sheath introduced over a previously placed J-wire guide (20,21) . The potential risk of contamination with oral flora is therefore minimised. This method of introduction is advantageous in patients with a fixed obstruction of the oesophagus or hypopharynx that prevents safe passage of a standard gastrostomy tube bumper.

A skin-level 'button' can replace the relatively more cumbersome gastrostomy tube once the gastrocutaneous fistula has matured (22). It is easy to insert and offers the advantage of greater comfort and cosmetic acceptability. Primary button gastrostomy placement has been described in recent publications with mixed results (23,24,25) and is in general not advisable.

Contraindications

To achieve success in PEG placement whilst avoiding serious complications, several safety precautions are important. Maximal gastric distension is the rule to bring the stomach in apposition with the abdominal wall so that indirect finger compression on the stomach is appreciated on endoscopic view. Endoscopic transillumination is necessary to ensure good placement of the cannula and to avoid the inadvertent creation of a gastrocolocutaneous tract. The anterior stomach wall should be free of any infection, inflammation or neoplastic disease. The first PEGs were placed at the junction between the middle two-thirds and outer one-third of a line drawn from the umbilicus to the left inferior costal margin.

The procedure is not recommended in the presence of a large diaphragmatic hernia, ascites or previous laparotomy. The presence of sepsis or multi-system organ failure would also preclude PEG placement.

Complications

PEG placement is virtually always possible. It is well accepted to be a safe and relatively easy procedure. Cumulative results from larger series show that the procedure-related mortality is low at less than 1%. Major complications occur in about 3% (1 - 7%) of patients. In order of frequency, these include peritonitis, bronchopulmonary aspiration, gastric perforation and gastric haemorrhage. The occurrence of major complications, namely aspiration and peritonitis lead to death in 25% of cases. Measures to prevent aspiration include the avoidance of over-sedation and airway maintenance

throughout the procedure. As there could be a transient ileus following the procedure, bowel sounds should be auscultated prior to the commencement of feeding. The patient should be propped up during feeding and this position should be maintained for 1 hour after the feeding has stopped. Early recognition and prompt treatment with broad-spectrum antibiotics will minimise untoward outcomes in the majority of patients (11,26,27).

The commonest cause of peritonitis is premature dislodgement of the gastrostomy tube which occurs in 0.8 to 3.4% of PEG patients (3,11,26) with the risk of gastric perforation. There should be no tension when the gastrostomy tube bumper is placed in contact with the gastric mucosa and the outer bolster is only loosely in contact with the skin. This avoids tissue necrosis and therefore tube extrusion and reduces the risk of leakage of gastric contents into the peritoneal cavity. Again, emphasis should be placed on early detection and appropriate treatment which includes exploratory laparotomy if warranted.

Minor complications occur in about 8% of cases. The most common minor complication is peristomal wound infection. Other complications include stomal leak, dislodged tube, tube migration and ileus.

Epilogue

PEG is in general, a relatively simple endoscopic procedure which offers significant palliation to patients. However it is still a procedure that is not widely known or accepted by doctors in various specialities who continue to keep nasogastric tubes in patients for long periods of time at great discomfort and inconvenience to patients.

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ORIGINAL ARTICLE

MULTIPLE RECEPTOR SITES IN *ACHATINA FULICA FERUSSAC* NEURONS FOR A MOLLUSCAN TETRAPEPTIDE AMIDE (FMRF-AMIDE)

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ABSTRACT: The effects of Phe-Met-Arg-Phe-NH_2 (FMRF-amide) and its analogue Phe-Leu-Arg-Phe-NH_2 (FLRF-amide) on the nervous system were studied on thirty-four identified neurons of the snail *Achatina fulica Ferussac* (locally known as "siput babi"). The results showed that FMRF-amide and FLRF-amide induced three different types of responses on the membrane potential of these neurons. First, FMRF-amide and FLRF-amide induced slow hyperpolarizing responses in TAN, d-LCDN, RAPN, d-LPeCN and V-RPLN. These responses remained unchanged in Cl^--free, Ca^{2+} -free and 20% Na^+ saline. The hyperpolarization was slightly reduced in 200% K^+ and slightly enhanced in 50% K^+ saline. Thus, the slow hyperpolarizing responses induced by FMRF-amide and FLRF-amide in the above neurons were K^+ -dependent.

Second, FMRF-amide and FLRF-amide induced rapid and transient hyperpolarizing responses on RPeNLN, LPeNLN and TAN-2. The effect persisted in 200% K^+, 20% Na^+ and Ca^{2+} -free saline. However, in Cl^--free saline, the effects were transient rapid depolarizing responses. This observation suggested that Cl^- was responsible for the hyperpolarizing responses.

Third, FMRF-amide and FLRF-amide induced depolarizing responses in INN and this effect remained unchanged in Ca^{2+} -free saline. However, in 20% Na^+ saline, the depolarizing responses were abolished. Thus, the involvement of Na^+ was implicated in the observed depolarizing responses.

The observed 3 different responses induced by FMRF-amide and FLRF-amide on *Achatina fulica Ferussac* neurons were comparable to their effects on the neurons of the gastropods *Aplysia* and *Helix*. Thus, FMRF-amide induced its multiple effects via multiple receptor sites, analogous to its action on *Helix* and *Aplysia* neurons. *(JUMMEC 1996: 1(2): 33-38)*

KEYWORDS: FMRF-amide, Neuropeptide, multiple receptor sites

Introduction

Biologically active neuropeptides are frequently used as extracellular chemical mediators in the central nervous system (1). Various neuropeptides have been found in the central nervous system of both vertebrates and invertebrates and many of these substances are widely thought to be potential neurotransmitters (1,2,3,4). Neuropeptides have a variety of distinctive characteristics that distinguish them from the more classical neurotransmitters. For instance, the localization of action of these peptides are often not restricted to synapses. In addition, most neuropeptides are synthesized as part of large precursors in the cell soma, whereas small molecule neurotransmitters are more often synthesized in the nerve terminals (5,6,7). Single neurons have been shown to utilize more than one chemical mediator, often in combination, consisting of a classical neurotransmitter and one or several other neuropeptides (8).

Phe-Met-Arg-Phe-NH_2, a tetrapeptide known as FMRF-amide isolated from the ganglia of the clam *Macrocalista nimbosa* (9), exhibits a potent pharmacological action on cardiac and non-cardiac muscle and on various neurons of several molluscan species (10,11). Electrophysiological investigations on the large neurons of gastropods such as *Aplysia* (12,13,14,15) and *Helix* (16,17) have indicated that FMRF-amide is capable of

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modifying in various ways the electrical properties of the neuronal membrane. However, such observations are not reported in the neurons of *Euhara*, a Japanese domestic snail (18). The responses induced varies depending on the specific neuron investigated. The responses include fast Cl^--dependent hyperpolarizing responses, slow K^+-dependent hyperpolarizing responses and depolarization responses that could be attributed to influx of sodium/calcium ions or suppression of voltage-dependent calcium-activated potassium current (19).

The snail, *Achatina fulica Ferussac*, was used in the present study. On the basis of localization, axonal pathways and resting membrane potential characteristics, Takeuchi *et al* (20) had identified 34 giant neurons in the ganglia of this specie of invertebrates. The large (500 μm) and easily identifiable neurons of this species provide an excellent experimental neural system model for electrophysiological research on the mechanisms of action of neurotransmitters, neuropeptides or neurotoxin.

The action of FMRF-amide and its naturally occurring analogue peptide FLRF-amide (Phe-Leu-Arg-Phe-NH_2) were investigated on these 34 identified *Achatina* neurons. To tentatively identify the ionic species and channels responsible for the responses, the experiments were repeated following changes in the ionic composition of the bathing medium.

Materials and Methods

The snails, *Achatina fulica Ferussac*, were collected and kept at room temperature between 22-25 °C until required. The snail neurons were prepared according to the method of Sun and Takeuchi (21). Briefly, the snail ganglia were exposed and the outer layers of connective tissue protecting the ganglia were removed manually until the last layer. The tissues enclosing the neurons were then softened with 0.7% trypsin (Type III, Sigma Chemicals, U.S.A.) at room temperature (25° ± 1°C) for 15 minutes. The ganglia were pinned onto a sylgard layer in a 0.2 ml experimental chamber and the remaining tissues were carefully removed with sharp tweezers to expose the neuron soma.

Conventional electrophysiological recording techniques were used to record the intracellular membrane potentials. Recording glass microelectrode were filled with filtered 3M potassium acetate (adjusted to pH 6.8). Electrode resistance measured ranged between 8-30 MΩ. Thirty-four identified neurons of *Achatina fulica Ferussac* (Figure 1A & 1B) were tested.

Saline flow to the tissue chamber was maintained at a rate of 4 ml per minute. Prior to the application of the FMRF-amide or FLRF-amide (Sigma Chemicals, U.S.A) the perfusion was stopped and 0.6 ml of saline were applied into the experimental chamber as control (no changes in the neuronal membrane potential). Later 0.6 ml of FMRF-amide or FLRF-amide dissolved in the appropriate solution to 10^{-4} M were directly applied into the chamber at a speed of 0.6 ml / 5-8 s. Any changes in the properties of the neuron membrane potential were detected via a two-channel pulse code modulation analogue to digital (PCM A/D) adapter (Medical System, U.S.A) and recorded by a video recorder. A hard copy was obtained through the play back facility of the MacLab System (Macintosh) via the PCM A/D adapter.

Normal saline had the following composition : NaCl 65.6 mM, KCl 3.3 mM, $CaCl_2$ 10.7 mM, $MgCl_2$ 13.0 mM, Tris HCl 9.0 mM and Tris Base 1.0 mM with pH adjusted to 7.5 (22). The extracellular K^+ and Ca^{2+} were augmented by simple addition of KCl or $CaCl_2$. The reduction of the extracellular Na^+, K^+, Cl^-, Ca^{2+} were made by replacing the ions with Tris. Ca^{2+}-free solution was made by replacing Ca^{2+} with Co^{2+}. Ca^{2+}-free solution enriched (3x) with Mg^{2+} was used to reduce trans-synaptic events.

Results

Neuronal electrical membrane responses to FMRF-amide and FLRF-amide on thirty-four identified Achatina *fulica Ferussac* neurons (Figure 1A and 1B) could be characterised into three main classes:

1. *Slow hyperpolarizing responses caused by both FMRF-amide and FLRF-amide.* These responses were exhibited by five identified neurons - TAN, d-LCDN, RAPN, d-LPeCN and V-RPLN. This class of responses is characterized by a slow onset (few seconds) and long lasting hyperpolarization. Examples of the slow hyperpolarizing response on TAN to FMRF-amide (Figure 2A) and FLRF-amide (Figure 3B) are shown. The responses were attenuated in 200% K^+ (FMRF-amide: Figure 2B; FLRF-amide: Figure 3B), and slightly enhanced in 50% K^+ saline (FMRF-amide: Figure 2C; FLRF-amide: Figure 3C). In Ca^{2+}-free saline enriched with 3 times Mg^{2+} and 20% Na^+ saline, the hyperpolarizing responses to FMRF-amide and FLRF-amide remained unchanged.

2. *Fast and rapid hyperpolarizing responses.* This class of induced responses by FMRF-amide and FLRF-amide lasted only a few seconds and were only observed in three identified neurons- RPeNLN, LPeNLN and TAN-2. Examples of the fast and rapid hyperpolarizing responses induced by FMRF-amide and FLRF-amide on LPeNLN are shown in tracing 4A and 4B. In Cl^--free saline, FMRF-amide (Figure 4C) and FLRF-amide (Figure not shown) induced a fast depolarization (compare the response in normal saline). In Ca^{2+}-free saline enriched

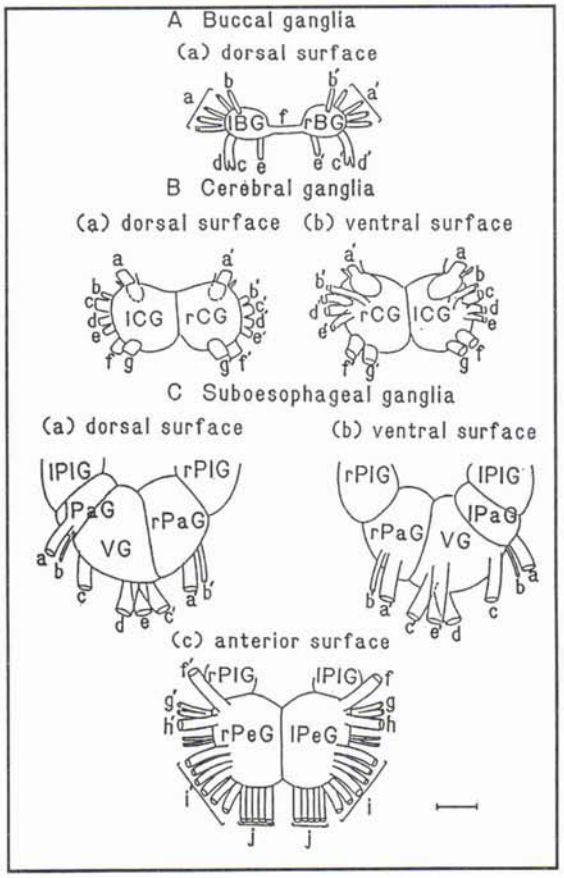

Figure 1A

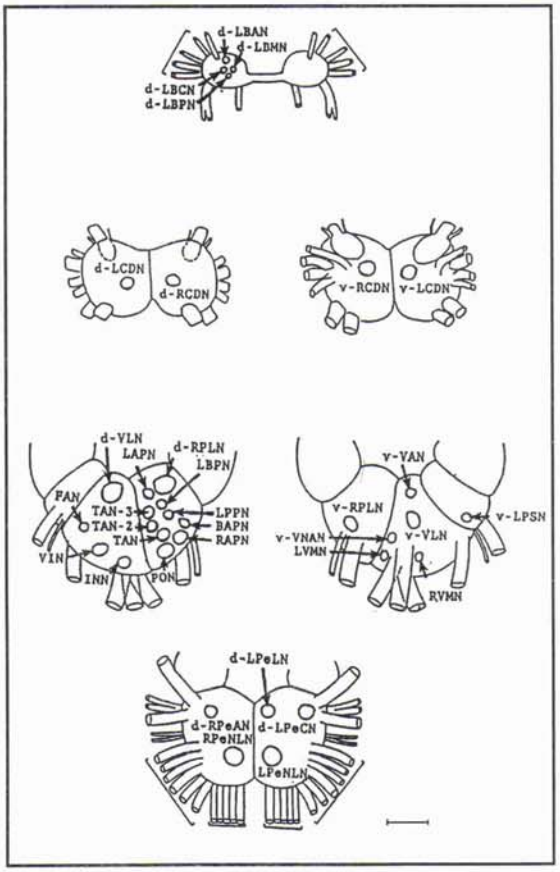

Figure 1B

Figure 1A:

Schematic drawing of the three ganglia of Achatina fulica Ferussac. A. buccal ganglia (dorsal surface). lBG. left buccal ganglion: rBG right buccal ganglion. B. cerebral ganglia. (a). dorsal surface; (b) ventral surface. lCG. left cerebral ganglion; rCG. right cerebral ganglion. C. suboesophageal ganglia. (a). dorsal surface; (b). ventral surface; Cc). anterior surface. 1PlG. left pleural ganglion; lPaG. left parietal ganglion; VG. visceral ganglion; rPaG. right parietal ganglion; rPlG right pleural ganglion; rPeG. right pedal ganglion; lPeG left pedal ganglion. Horizontal bar: scale (500 μm).

Figure 1B:

Identification of giant neurons.

A. *Buccal ganglia (dorsal surface). d-LBAN (dorsal-left buccal anterior neuron) d-LBMN (dorsal-left buccal medial neuron) d-LBCN (dorsal-left buccal central neuron) : d-LBPN (dorsal-left buccal posterior neuron).*
B. *Cerebral ganglia. (a). dorsal surface. d-LCDN (dorsal-left cerebral distinct neuron) d-RCDN (dorsal-right cerebral distinct neuron). (b). ventral surface. v-LCDN (ventral-left cerebral distinct neuron) v-RCDN (ventral-right cerebral distinct neuron).*
C. *Suboesophageal ganglia. (a). dorsal surface. d-VLN (dorsal visceral large neuron) FAN (frequently autoactive neuron) VIN (visceral intermittently autoactive neuron) INN (intestinal nerve neuron) PON (Periodically oscillating neuron) TAN, TAN-2; TAN-3; (tonically autoactive neuron) RAPN (right anterior pallial nerve neuron) d-RPLN (dorsal-right parietal large neuron) LAPN (left anterior pallial nerve neuron) LBPN (left bifurcate pallial nerve neuron) LPPN (left posterior pallial nerve neuron) : BAPN (bilateral anterior pallial nerve neuron). (b). ventral surface. v- LPSN (ventral- left parietal silent neuron) VLN (ventral - visceral large neuron) v-VAN (ventral -visceral anterior neuron. old name: v-l-VOrN) LVMN (left visceral multiple spike neuron. old name: l-VMN) RVMN (right visceral multiple spike neuron. old name: r-VMN); v-VNAN (ventral-visceral noisy autoactive neuron) : v-fRPLN (ventral right parietal large neuron). (c). Anterior surface. d-LPeLN (dorsal-left pedal large neuron) LPeCN (dorsal-left pedal constantly firing neuron) LPeNLN (left pedal nerve large neuron) : d-RPeAN (dorsal right pedal autoactive neurone) PeNLN (right pedal nerve large neuron). Horizontal bar scale (500 μm).*

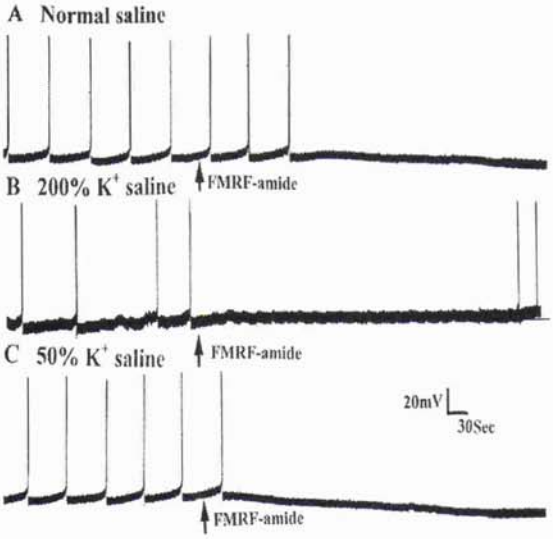

Figure 2. *The effect of FMRF-amide inducing a slow hyperpolarizing response on TAN (Figure 2A). The responses were slightly reduced (shortened) in 200% K^+ (Figure 2B) and slightly enhanced in 50% K^+ saline (Figure 2C).*

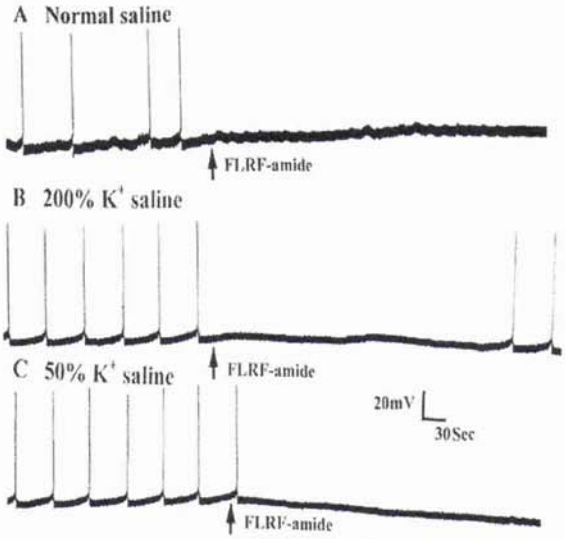

Figure 3. *The effect of FLRF-amide inducing a slow hyperpolarizing response on TAN (Figure 3A). The responses were slightly reduced (shortened) in 200% K^+ (Figure 3B) and slightly enhanced in 50% K^+ saline (Figure 3C).*

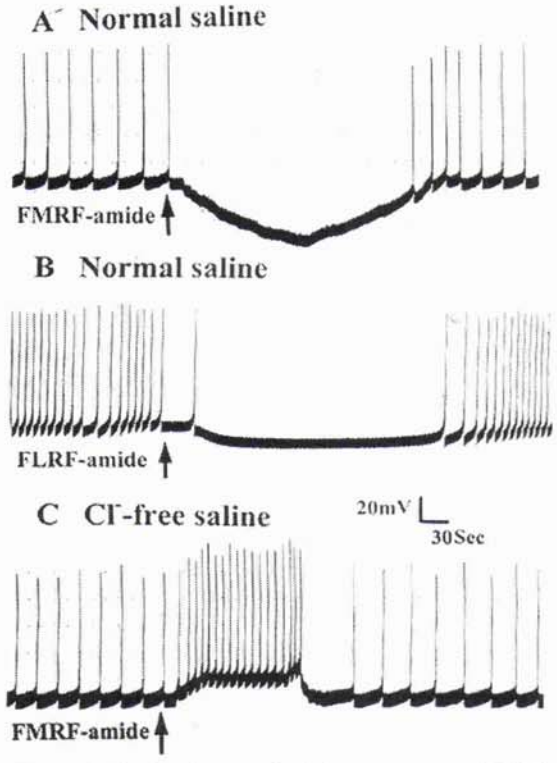

Figure 4. *The fast hyperpolarizing response to FMRF-amide (Figure 4A) in LPeNLN . A similar effect was obtained with FLRF-amide. (Figure 4B). In Cl^--free saline, FMRF-amide caused depolarization (Figure 4C; contrast with the response with normal saline).*

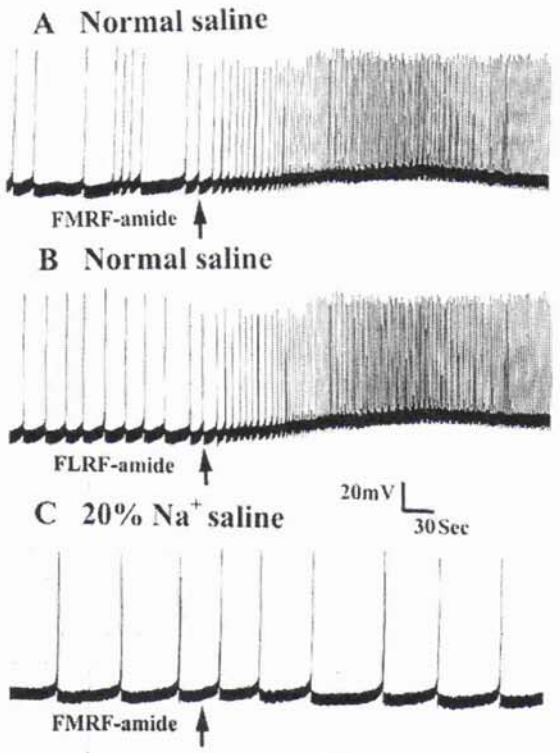

Figure 5. *The fast and rapid depolarizing response to FMRF-amide on INN(Figure 5A). FLRF-amide when given directly into INN induced a rapid depolarizing response similar to FMRF-amide (Figure 5B). In 20% Na^+ saline, the excitatory effects of FMRF-amide was abolished (Figure 5C). A similar response was obtained with FLRF-amide.*

with 3 times Mg^{2+}, 20% Na^+ saline, 200% K^+ and 50% K^+ saline, the responses to FMRF-amide and FLRF-amide remained unchanged.

3. *Fast and rapid depolarizing responses induced by FMRF-amide and FLRF-amide were characterized by fast onset and long-lasting excitatory effect.* This type of response could only be observed in INN (Figure 5 and 5B). These responses remained unchanged in Ca^{2+}-free saline enriched with 3 times Mg^{2+} or Cl^--free saline, 200% K^+ saline or 50% K^+ saline. However, in 20% Na^+ saline, the FMRF-amide fast depolarization was abolished (Figure 5C). A similar result was seen with FLRF-amide.

Discussion

The molluscan neuropetide Phe-Met-Arg-Phe-NH_2 (FMRF-amide) was discovered in the ganglia of the clam *Macrocallista nimbosa* by Price and Greenberg (9). FMRF-amide was initially found to be a cardioexcitatory neuropeptide, but it was later discovered to have a potent pharmacological action on a range of cardiac and non-cardiac muscles as well as neurons of different species of mollusc (10,11). Extensive studies on the action of FMRF-amide had been carried out on *Aplysia* and *Helix* neurons. Multiple actions of FMRF-amide were reported on *Helix* neurons (17) leading to the identification of multiple receptor sites for FMRF-amide on Helix neurons (19). Multiple actions of FMRF-amide on *Aplysia* neurons were published by Stone and Mayeri (12) and FMRF-amide was believed to act as a neurotransmitter, neuropeptide or neurohormone in *Aplysia* (23). Price (11) discovered two other naturally occurring analogues of FMRF-amide (FLRF-amide and pQDPFLRF-amide) in the same clam.

The data from these experiments support the hypothesis that FMRF-amide has multiple actions and acts on spatially different receptor sites. At least three different classes of actions were identified in *Achatina* neurons. First, slow inhibitory responses induced in five neurons - TAN, d-LCDN, RAPN, d-LPeCN and v-RPLN (see Figure 1a and 1b for location and identity of neurons). Second, rapid and transient inhibition on three neurons- RPeNLN, LPeNLN and TAN-2. Third, fast and rapid depolarization observed only in INN. Similar, responses in the same identified neurons were observed when another naturally occurring analogue of FMRF-amide, FLRF-amide, was used. There is a strong possibility that both FMRF-amide and its analogue, FLRF-amide, mediate their responses via the same receptors. However, the receptor species vary with different identified neurons leading to different classes of neuronal membrane characteristics. Tentative conclusions relating to the specie of ions and type of ionic channel involved could be derived from experiments where different ionic concentrations of different ionic species were changed in the bathing mediums. The results of these types of experiments suggest that the neuropeptide induces at least three different ionic mechanism on *Achatina fulica Ferussac* neurons. Based on their induced responses, FMRF-amide and its analogue FLRF-amide acted either as inhibitory or excitatory neurotransmitter or neuropeptide in the *Achatina* neural system. On the basis of the population of the responses, the amides are believed to serve mainly as inhibitory rather than excitatory neurotransmitters or neuropeptide. The results clearly show that FMRF-amide and FLRF-amide receptors are specifically localized, since the induced responses were only found on certain neurons. These neurons may be equipped with specific ionic mechanisms which were not abolished in Ca^{2+}-free Mg^{2+} enriched (3x) saline. This observation clearly indicates that the responses observed could not be attributed to trans-synaptic input.

Both FMRF-amide and FLRF-amide induced slow hyperpolarization in five neurons of *Achatina fulica Ferussac*. The onset of the response occurred a few seconds after introduction of both amides and could last for a few minutes. These responses were enhanced in 50% K^+ saline indicating enhanced K^+ efflux out of cell along the K^+ concentration gradient. When extracellular concentration of K^+ was increased to 200%, responses to both amides also decreased. Changing extracellular composition of the bathing milieu to Cl^--free, Ca^{2+}-free and 20% Na^+ salines did not affect the responses to the amides. Thus, the slow inhibitory responses in the presence of FMRF-amide and FLRF-amide could tentatively be attributed to the opening of K^+ channels. FMRF-amide induced slow hyperpolarization responses in association with K^+ was also reported in *Aplysia* (12) and *Helix* neurons (17).

Fast and rapid hyperpolarizing responses to both amides in three identified *Achatina* neurons could be attributed to the opening of Cl^- channels. Influx of the Cl^- ions into the cell via these receptors could be responsible for the observed hyperpolarization. The hypothesis is augmented with the observation that the initial rapid hyperpolarizing responses could be reversed, showing rapid depolarization when extracellular of Cl^- was removed. Chloride channels opened by FMRF-amide and FLRF-amide in this instance would cause an efflux of the Cl^- ions and thus depolarization of membrane potential. Similar results were also obtained in *Helix* neurons (17).

Finally, only one identified neuron of *Achatina fulica Ferussac* responded with depolarization in the presence of FMRF and FLRF-amide. This response was sensitive only to reduction in Na^+ ions. The ionic basis of these depolarizing responses is in concurrence with *Helix* neurons (17) and *Aplysia* neurons (12).

In conclusion, FMRF-amide is capable of inducing three different kind of receptor responses on *Achatina fulica*

Ferussac neurons, possibly with involvement of spatially distinctive receptor sites and species.

Acknowledgements

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ORIGINAL ARTICLE

ULCERATIVE COLITIS IN MALAYSIANS

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ABSTRACT: **A review of all colonic biopsies received by the Department of Pathology during a 8-year period revealed 41 cases of ulcerative colitis (UC). The diagnosis was based on histological and clinical features. The age range of patients was between 14 - 76 years with a median age of 35.4 years. The disease was more prevalent among Indians. The common presenting symptoms were diarrhoea (100%) and haematochezia (83%). The extent of colonic involvement varied. Twelve patients (29.2%) had pancolitis and 8 (19.5 %) had proctitis. Extraintestinal manifestations were rare and only one patient had pyoderma gangrenosum. One patient developed multifocal colorectal cancer 10 years after the inial diagnosis of UC and died 2 years later due to metastases. Histology plays an important role in the diagnosis and management of patients with UC. We noted a good correlation between clinical and pathological features. The most recent colonic biopsy showed features of chronic UC with activity in 34 cases and features of remission in 4 cases.** *(JUMMEC 1996 1(2): 39-42)*

KEY WORDS: **Ulcerative colitis, Malaysians, pathology**

Introduction

Ulcerative colitis (UC) is an idiopathic chronic inflammatory bowel disease which affects the mucosa of the large bowel Although it is an uncommon disease in Malaysia, it should be considered in the differential diagnoses of colitis. Careful histological examination of the colonic biopsy plays an important role in the diagnosis and management of patients with UC. This is a clinicopathological study of histologically proven cases of UC in the University Hospital over a period of 8-years.

Materials and Method

Histological sections of all colonic biopsies received by the Department of Pathology, University Hospital, Kuala Lumpur between January 1986 and December 1993 were reviewed. Special stains such as periodic acid Schiff were done when necessary to rule out parasitic infection. Clinical information was obtained from the request forms accompanying the biopsies and from the patient records.

The final diagnosis of UC was based on the clinical features, colonoscopic findings, histology and stool examination and culture, and in some cases barium enema findings.

Multiple biopsies were done in patients when the clinical diagnosis of relapse, dysplasia or infection was considered.

Results

Colonic biopsies of 41 patients with UC were received between 1986 and 1993, of which 14 cases had been diagnosed to have UC earlier based on clinical and histological findings. There were 24 males with a male to female ratio of 1:0.7.The age range of patients at the time of initial clinical presentation was between 14 to 76 years and the median age was 35.4 years.

The racial distribution of patients was as follows: 17 Chinese (41.5%), 8 Malay (19.5%) and 16 Indians (39.0%). Indians formed 27.0 % of the Hospital attendance of the University Hospital during the period of study. Compared with these baselines (Table 1), there appears to be a higher prevalence of UC among Indians ($p < 0.01$).Table 1 shows the distribution of cases with UC and the total number of patients seen in the University Hospital during the study period with regard to ethnic distribution.

Clinical features

All the patients presented with a history of diarrhoea. The diarrhoea was bloody in 34 (83%) and mucoid in 15 (36.5 %) patients respectively. Twelve patients (29.3%) had total colitis and 8 (19.5%) had only proctitis. The disease involved the recto - sigmoid region in 9

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patients (21.9%) and extended up to the splenic flexure in 12 patients (29.3 %). Only 1 patient had extraintestinal manifestations such as pyoderma gangrenosum. None of the patients had arthritis or involvement of the hepatobiliary system. The duration of the clinical symptoms was 2 weeks (3 cases), 1 to 2 months (28 cases), 3 months to 1 year (4 cases), and 1 to 3 years (5 cases). One patient presented at the age of 29 years with a 12-year history of bloody diarrhoea.

Table 1: Ethnic distribution of patients with Ulcerative colitis (UC) and total Hospital admissions between 1986-1993.

	Chinese	Malays	Indian
	No (%)	No (%)	No (%)
Patients with UC	17 (41.5)	8 (19.5)	16 (39)
Hospital admissions	63,924 (29.5)	91,444 (42.2)	58,507 (27)

$p < 0.01$

A female patient presented at the age of 15 years with a one -year history of bloody diarrhoea. Biopsy showed UC. She was on treatment and was on regular follow-up for eight years . Three biopsies were done during this period and showed active, chronic colitis. She did not attend the clinic for 2 years subsequently and consulted the physician again at the age of 25 years for bloody diarrhoea. Colonoscopy at that time showed tumours at the caecum, transverse and descending colon. At surgery, moderately differentiated adenocarcinoma was noted at the sites, invading up to the serosa with spread to regional lymph nodes (Dukes C). Two years following colectomy, she had bilateral Krukenberg tumours and succumbed within a year.

Complications

Two patients were treated with colectomy, one of whom had severe colitis resistant to treatment and the other suffered accidental perforation during colonoscopy.

Pathology

There was a good correlation between clinical, endoscopic and histological findings in all cases in this study. Microscopic features in the most recent biopsy in 38 cases showed chronic UC with activity in 34 cases and chronic UC in remission in 4 cases. The common findings in active disease were crypt abscess (Fig 1), crypt loss and crypt distortion (Fig 2). The histological findings are summarised in Table 2.

Table 2. Histological features observed in 38 cases of UlcerativeColitis

	No	%
Chronic UC with activity (34 cases)		
Focal mucosal ulceration	30	88.2
Inflammation of crypts cryptitis and abscess	31	91.1
Crypt distortion	31	91.1
Mucus depletion	28	82.3
Inflammatory infiltrate in lamina propria (polymorphs, lymphocytes and plasma cells)	34	100
Villous metaplasia	11	32.3
Inflammatory polyp	8	23.5
Severe dysplasia	0	0.0
Chronic UC in remission (4 cases)		
Crypt distortion	4	100
Inflammation in crypts and lamina propria	0	0.0
Metaplasia	0	0.0
Dysplasia	0	0.0

Discussion

Ulcerative colitis (UC) , is still an uncommon disease in Malaysia. Only a few reports from the local population have been published (1,2)

In this study, we noted that Indians appear to be more commonly affected when compared with the other races. Similar observations were noted in a Malaysian study done by Thien-Hut (2) and neighbouring Singapore (3). An epidemiological study done by Probert *et al* (4) showed that South Asians in Leicestershire in England have twice the risk for UC compared with Europeans. The risk was greatest in Hindus and Sikhs. In our study, of the 16 cases of Indians, 15 were Hindus and 1 was Sikh. The role of diet and genetic factors in the causation of UC has to be investigated as a possible aetiological factor for the higher incidence of UC among Indians.

The aetiology of UC remains unknown. There has been no evidence of a causal relationship between any microbial agent and UC. Research studies indicate that UC is an autoimmune disease. Autoantibodies to perinuclear cytoplasmic components of neutrophils (pANCA) is found in 60-70% of patients with UC (5). Yang *et al* (6) have noted an association between UC

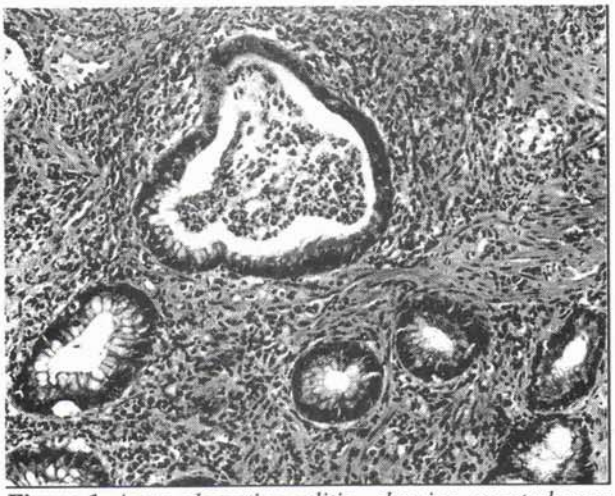

Figure 1. *Acute ulcerative colitis , showing crypt abscess and inflammation of lamina propria. Haematoxylin and eosin X 200.*

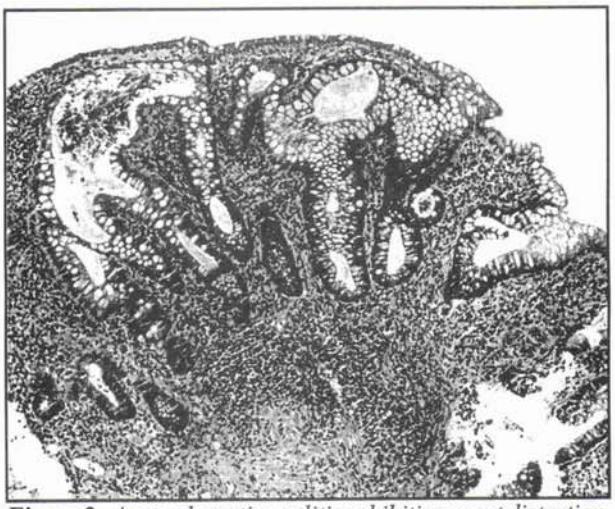

Figure 2. *Acute ulcerative colitis exhibiting crypt distortion. Haematoxylin and eosin X 40.*

and HLA-DR2 antigen. Patients suffering from UC who are HLA-DR 2 positive are likely to have pANCA antibodies. Thus it appears that pANCA autoantibodies identify a group of UC patients with a genetic predisposition. An increased level of immune stimulation in patients with inflammatory bowel disease (IBD) has been documented. Patients with IBD have a high number of activated T lymphocytes in the lamina propria of the intestine. T cells have the capacity to perform a variety of activities upon contact with pathogens and in healthy people, resulting in clearance of the pathogen. Probably, T lymphocytes in patients with BD have qualitative and/or quantitative disturbance in antigen specificity and reactivity to microbial antigen. As a result, exposure to bacterial antigens in the gut lumen causes intestinal inflammation (7).

The differentiation of UC from infective colitis poses a problem to the clinician. Patients with UC usually have an insidious onset and present with diarrhoea. Infective colitis is generally characterised by an acute onset of bloody diarrhoea, high fever and abdominal cramps (8). However, concurrent infection, traveling abroad and treatment with antibiotics may alter the initial symptom of UC to more acute infectious colitis like features. This is due to alteration of the intestinal flora. Stool culture should be done in all cases of diarrhoea. In published studies, positive stool culture was found in 50 - 73% of patients with acute, presumably infectious diarrhoeal disease (9,10). Sigmoidoscopy may not differentiate UC from infective colitis with any degree of certainty. Thus histopathology is a reliable diagnostic tool for the differentiation of UC from infective colitis (11). The features of UC can be found as early as one week in a first attack of UC and these include plasma cell infiltration in the lamina propria extending up to the mucosal base and crypt changes such as crypt distortion and crypt atrophy (12). The differentiation of UC from Crohn's disease should be based on all evidence including clinical, radiological, endoscopic findings and histology (13). In our study, none of the patients with UC had epitheloid cell granuloma and all biopsies showed diffuse inflammation. The final diagnosis was a correlative clinico-pathological one.

It is well known that there is an increased risk of colorectal carcinoma in patients with UC. The risk is greatest among patients with pancolitis. The cumulative possibility of developing carcinoma is about 3% at 15 years and 5% at 20 years (14) . Colonoscopy with biopsies is advisable at regular intervals after the duration of disease reaches 10 years. Dysplasia is a marker of malignant potential. However, it is a descriptive finding and subject to intra and inter observer variation (15). Dysplasia is patchy and small biopsy specimens may not show it. The most reliable clinical marker of dysplasia is a proliferative lesion such as a villous or polypoid area on endoscopy (16). A single report of low grade dysplasia (mild) is an indication for increased vigilance. Patients with high grade dysplasia in a proliferative lesion should be advised surgery (14). In our study, none of the cases showed high grade dysplasia. Nine patients in our study, with UC of more than 10 years duration are on regular follow-up and a recent biopsy in all these cases showed no severe dysplasia.

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ORIGINAL ARTICLE

MANAGEMENT OF BLUNT LIVER INJURIES: ROLE OF THE CONSERVATIVE APPROACH.

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ABSTRACT: Abdominal CT scanning makes non-operative management of liver injury possible. We reviewed medical records of 112 blunt trauma patients with heeatic injury who received initial abdominal CT scan. We examined: 1) Indications for delayed surgery, 2) Disposition or cause of death, 3) Results of follow-up CT scans, 4) Long-term complications. Over a 5-year period, 1397 patients were admitted for blunt trauma, of which 152 patients were found to have hepatic injury. Forty patients presented either clinically unstable or with an acute abdomen and underwent diagnostic peritoneal lavage or immediate laparotomy without a CT scan. Abdominal CT scan was performed on 112 patients, 38 of whom had hepatic injury or associated major abdominal injury and underwent laparotomy. Two patients died of cardiac arrhythmias following CT scanning. The remaining 72 patients received initial non-operative management of their hepatic injury. Six patients in this group underwent delayed abdominal surgery. Four developed acute abdomen. Two had planned nephrectomies. No patient required surgical treatment of the liver injury at the time of laparotomy. Eight deaths occurred in the 72 patients managed non-operatively, all due to associated extra-abdominal injuries. Thirty-eight patients had 54 CT scans taken as follow-up examination at intervals of 1 to 94 days post-injury. All of the CT scans showed stabilisation or improvement of hepatic injury. Six patients who had CT scans taken at 3 months post-discharge were asymptomatic, with radiological resolution of their hepatic injury. Thirty-eight patients were followed for an average of 61.8 days (range 7-203 days) after discharge with no complications from liver injury. We conclude that non-operative management of blunt hepatic injury is an appropriate option in selected patients, and that long-term follow up CT scans may not be necessary in asymptomatic patients. *(JUMMEC 1996 1(2): 43-48)*

KEYWORDS: Liver, blunt trauma, non-operative

Introduction

The liver is the most frequently injured abdominal organ following blunt trauma. Major hepatic injury accounts for significant mortality, usually due to severe hemorrhage (1,2). Many hepatic injuries found at laparotomy are not actively bleeding and require no operative treatment (2-5). Abdominal CT scanning plays an increasing role in the initial evaluation of the haemodynamically stable patient with blunt abdominal trauma. CT scanning allows the specific diagnosis of many abdominal injuries which otherwise would go undetected, or would require laparotomy to rule out life threatening injuries. Many authors advocate non-operative management of selected groups of clinically stable patients with isolated hepatic injury diagnosed by CT scan (1,3,6-11).

In order to evaluate the outcome of non-operative management of blunt hepatic trauma and the role of CT scanning in the initial evaluation, we retrospectively reviewed 112 patients with blunt abdominal trauma who had hepatic injury diagnosed by an initial CT scan.

Methods

The in-patient records at the University Hospital Kuala Lumpur, Malaysia were reviewed for all blunt trauma patients with hepatic injury admitted to the surgical wards from January 1991 to December 1995. We examined indications for delayed surgery, disposition or cause of death, results of follow-up CT scans, and long-term complications.

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The severity of the liver injury was classified according to a useful classification developed by Shackford and colleagues (American Association for the Surgery of Trauma - Hepatic Injury Scale - Table 1)(14).

Table 1. Management classification for civilian hepatic trauma (Shackford)

Grade	Liver injury*
I	Capsular avulsion Parenchymal fracture < 1 cm deep
II	Parenchymal fracture 1-3 cm deep Subcapsular haematoma < 10 cm deep Peripheral penetrating wound
III	Parenchymal fracture > 3 cm deep Subcapsular haematoma > 10 cm in diameter Central penetrating wound
IV	Lobar tissue destruction Massive central haematoma
V	Retrohepatic vena cava injury Extensive bilobar disruption

* CT scan is used to obtain a pre-operative estimation of the injury grade. The liver injury is usually obvious following inspection or palpation at laparotomy. Capsular tears, minor parenchymal tears, simple stab wounds and low velocity gunshot wounds constitute well over half of all liver injuries and most have stopped bleeding by the time of exploration. If still oozing, capsular injuries can be controlled by mild direct pressure, with or without suturing, or by electrocautery. Sometimes topical haemostatic agents such as microfibrillar collagen may be helpful. Post-operative drainage is unnecessary for these superficial injuries except when the degree of injury present suggests a distinct possibility of post-operative bleeding or bile leak, in which case closed suction drainage is instituted and maintained as long as drainage occurs. The surface of most of these injuries should not be tightly closed, as pocket of blood or bile may collect and result in an intrahepatic abscess or subsequent haemobilia. However, in more severe cases, resection (either segmentectomy or hepatectomy) may be required to remove crushed liver tissue. Packing of the bleeding liver tissue (for 24-48 hours) is advised in the severely compromised patient, to achieve haemodynamic stability and correction of coagulopathy. Re-exploration and definitive procedure is performed when the patient has been stabilised.

Results

Over a 5-year period, a total of 1397 patients were evaluated by the trauma service following blunt trauma. One hundred and fifty-two of these patients were found to have hepatic injury. Forty patients presented with hypotension (systolic blood pressure less than 100) or had acute abdominal findings and underwent diagnostic peritoneal lavage or immediate laparotomy without a CT scan. The majority of these patients (77.5%) had grade IV liver injuries noted at laparotomy (Table 2). The remaining 112 patients received an abdominal CT scan as part of their initial evaluation (Table 3). Thirty-eight of these patients (33.9%) underwent immediate laparotomy based on hepatic injury, associated intra-abdominal injury, or large haemoperitoneum diagnosed by CT scan (Table 4). The liver injuries of these patients were estimated to be of grade III by pre-operative CT scan. However, in 16 patients (42.1%), the liver injuries were found to be more severe intra-operatively (grade IV) than estimated by CT scan. Twenty-three out of the 38 patients (60.5%) who underwent immediate laparotomy had liver resections (Table 4).

Two patients died in the emergency department from cardiac arrhythmias. The remaining 72 patients received initial non-operative management of their hepatic injury. The liver injuries in this group were either grades I or II (Table 3). Thirty-four males and 38 females made up this group, with a mean age of 31 years. The most common cause of trauma was a motor vehicle accident (Table 5). All but two of the 72 patients treated non-operatively had at least one major associated injury (Table 6). The most common extra-hepatic injuries were extremity fractures (40 patients), closed head injury (36 patients), and rib fractures (24 patients). Six patients in the non-operative group underwent delayed abdominal surgery at 48-72 hours later. Four developed an acute abdomen, and two had a planned nephrectomy (for persistent gross haematuria with decreasing haemoglobin level). No patient required surgical treatment of the liver injury at the time of laparotomy. Two of the four patients with an acute abdomen underwent cholecystectomy, and the other 2 had a negative laparotomy. Eight of the 72 patients managed non-operatively had died, each due to associated injuries (4 had died secondary to severe head injury, and 4 succumbed to respiratory failure). No patient had died as a result of the liver injury.

In the non-operative group (n=64 patients who survived), only 38 patients (59.4%) returned for follow-up (The remaining 26 patients were either from other states, uncontactable at their given addresses or had follow-ups at other hospitals). All these patients had 54 follow-up CT scans at intervals of 7 to 94 days post-discharge. All of the CT scans showed stabilisation or improvement of hepatic injury. Six patients who had CT scans at 3 months post-discharge showed complete radiological resolution of their injury. Of the 64 patients discharged from the hospital, 50 were discharged home, 8 were transferred to an acute rehabilitation facility, 4 were transferred to another hospital, and 2 left against medical advice. Thirty-eight patients were seen in follow-up, an average of 61.8 days (range 7-203 days) after discharge, with no long-term complications from liver injury.

Table 2. Intra-operative liver injuries noted in haemodynamically unstable patients (N=40) who underwent immediate laparotomy without a CT scan.

Liver injury (patients in subgroup)	Grade*	Number of patients
Parenchymal fracture > 3 cm deep (11) Subcapsula haematoma > 10 cm (20)	III	7 (17.5%)
Lobar tissue destruction (6) Massive central haematoma (1)	IV	31 (77.5%)
Retrohepatic vena cava injury	V	2 (5.0%)

*According to Shackford *et al* 1989(14)

Table 3 CT scan findings of 112 patients with liver injuries.

Liver injury (patients in subcatogery)	Grade*	Number of patients
Capsular avulsion (34) Parenchymal fracture<1cm (10)	I	44
Parenchymal fracture 1-3cm (6) Subcapsular haematoma<10cm (24)	II	30**
Parenchymal fracture >1-3cm (10) Subcapsular haematoma >10cm (15) Central penetrating wound (13)	II	30**

*Injury grade by CT scan estimation

**2 patients died of cardiac arrhythmias after CT scan

***These 38 patients underwent laparotomy after initial CT evaluation (Details in Table 4)

Table 4. Details of 38 patients who underwent laparotomy after initial CT scan evaluation.

Patient	Haemoperitoneum*	Associated injuries	Operative grade of liver injury	Liver operation
Parenchymal fracture (n=10)**				
1	++	Splenic tear	Same as CT	Suture
2	++	Perforated stomach	"	Suture
3	+++	Splenic tear	"	Suture
4	+	Splenic tear	"	Suture
5	+++	-	IV (Lobar tissue destruction)	(L) lateral segmentectomy
6	+++	Bowel perforation	IV (Massive central haematoma)	Resection segments V-VI
7	++	Splenic tear	Same as CT	Suture
8	+++	Perforated stomach	V (Lobar tissue destruction)	Resection segments VII-VIII
9	++	Splenic tear	Same as CT	Suture
10	++	Bowel perforation	Same as CT	Suture
Subcapsular haematoma (n=15)**				
11	+++	-	IV (Massive central haematoma)	Resection segments VI-VII
12	+	Bowel perforation	Same as CT	Nil (haematoma intact)
13	+++	Duodenal tear	Same as CT	Nil (haematoma intact)
14	+++	-	IV (Lobar tissue destruction)	(L) lateral segmentectomy
15	++	Splenic tear	Same as CT	Suture
16	+++	-	IV (Massive central haematoma)	Resection segments VI
17	+	Splenic tear	Same as CT	Nil (haematoma intact)
18	+++	-	IV (Lobar tissue destruction)	(L) lateral segmentectomy
19	+	Bowel perforation	Same as CT	Nil (haematoma intact)
20	++	Bowel perforation	Same as CT	Nil (haematoma intact)
21	++	Duodenal tear	Same as CT	Suture
22	++	Splenic tear	Same as CT	Suture
23	+++	Splenic tear	Same as CT	Suture
24	+++	Splenic tear	Same as CT	Suture
25	+++	Splenic tear	Same as CT	(L) lateral segmentectomy
Central penetrating wound (n=13)**				
26	+++	Splenic tear	Same as CT	Nil (haematoma intact)
27	+++	-	IV (Lobar tissue destruction)	(L) lateral segmentectomy
28	+++	-	IV (Lobar tissue destruction)	(L) lateral segmentectomy
29	+++	-	IV (Massive central haematoma)	Resection segments VI-VII
30	+++	Bowel perforation	Same as CT	Suture
31	+++	Perforated stomach	Same as CT	Suture
32	+++	Perforated stomach	Same as CT	Suture
33	+++	-	IV (Massive central haematoma)	Resection segments V-VI
34	++	-	IV (Massive central haematoma)	Resection segments V-VI
35	+++	-	IV (Lobar tissue destruction)	(L) lateral segmentectomy
36	+++	-	IV (Lobar tissue destruction)	(L) lateral segmentectomy
37	+++	-	IV (Lobar tissue destruction)	(L) lateral segmentectomy
38	+++	-	IV (Lobar tissue destruction)	Resection segments V-VI

*Haemoperitoneum – mild (+), moderate (++), large (+++)
**3 types of grade III liver injuries (n=38) noted at initial CT evaluation prior to laparotomy

Table 5. Group Characteristics

	Non-operative group	Total blunt trauma patients
Male:Female	1:1 (34/38)	3:1 (1048/349)
Mean Age	31(4-91 years)	34(0-94 years)
Causes		
Motor vehicle accident	64	823
Pedestrian	2	154
Fall	2	228
Others	4	326

Table 6. Associated Injuries

Chest (26 patients with 54 injuries)	
Hemo/pneumothorax	14
Flail chest/Rib fractures	24
Pulmonary contusion	6
Myocardial contusion	6
Sternal fracture	4
Abdomen (22 patients with 26 injuries)	
Renal injury*	14
Spleen injury	6
Duodenal injury (<1cm haematoma)**	2
Gallbladder injury**	2
Adrenal injury	2
Head or Spine (42 patients with 48 injuries)	
Closed head injury	36
Spine fracture	8
Facial fracture	4
Orthopaedic (46 patients with 54 injuries)	
Pelvic fracture	12
Extremity fracture	40
Shoulder dislocation	2
Soft Tissue (6 patients with 6 injuries)	
Complex laceration	4
Full thickness burn	2

*2 patients with renal injury had nephrectomies due to persistent gross haematuria

**These 4 patients underwent laparotomy for acute abdomen

Discussion

Hepatic trauma is responsible for significant mortality and morbidity among blunt trauma patients. Life-threatening liver injuries include large stellate fractures in the liver parenchyma, injuries involving the hepatic veins, retrohepatic vena cava, or vessels of the porta hepatis. These injuries can result in rapid exsanguination. However, subcapsular and intra-hepatic haematomas or simple lacerations are usually associated with little blood loss and may be managed non-operatively (2-5). Many workers advocate non-operative management of selected patients with liver lacerations found on CT scan (1,3,6-11). Selective non-operative treatment of liver laceration in children has been successful in the last few years(12) and is now becoming more popular in adult trauma. However, when management is non-operative, there is increased risk of delayed detection of associated abdominal injuries, primarily bowel perforation, that might not be revealed by CT scan. Therefore, some workers still advocate diagnostic peritoneal lavage (DPL) as the method of choice in evaluating abdominal trauma(13).

In the stable patient, the CT scan offers advantages over DPL. The CT scan is rapidly available, non-invasive and reveals the extent of hepatic and other abdominal injuries. It can define the extent of a haemoperitoneum or a retroperitoneal haematoma. DPL does not identify retroperitoneal injury or the source of intraperitoneal blood. It is also possible to produce a positive lavage with a relatively small amount of intraperitoneal blood (6). DPL continues to play a significant role in the evaluation when the patient is unstable, when a CT scanner is not readily available, in an uncooperative patient with a contraindication to sedation, or in a patient allergic to contrast agents(8). The choice of diagnostic procedures should be made on an individual basis, using the clinical information available.

Several workers advocate non-operative treatment in the haemodynamically stable patient with a benign abdominal examination and each of the following CT scan findings: single hepatic parenchymal laceration, intrahepatic haematoma, or subcapsular haematoma; no evidence of active bleeding; intraperitoneal blood less than 250mL (restricted to Morrison's pouch); absence of other intra-abdominal injury requiring surgery (7,8,15-18).

Our series showed that the majority of the liver trauma patients who were haemodynamically unstable had grade IV injuries (77.5%). CT scan findings of the liver injuries in the other 38 patients who underwent immediate laparotomy after the initial imaging are shown in Table 4. Pre-operatively, these patients were estimated to have grade III injuries. They had laparotomies based on presence of other abdominal visceral injuries (bowel

or stomach perforations, splenic tears) and presence of significant moderate to large haemoperitoneum (Table 4). Majority of these patients (24/38=63.2%) had associated injuries. However, a significant proportion of these patients (42.1%) had more severe injuries (grade IV) noted intra-operatively. In this group of patients, the pre-operative CT scan grading of liver injuries may not be accurate due to the presence of significant haemoperitoneum and pneumoperitoneum (due to bowel/gastric perforations) that could mask the actual liver injuries. Nearly a third of the parenchymal fractures of the liver (3/10 patients=30%), 26.7% (4/15 patients) of the subcapsular haematoma and 69.2% (9/13 patients) of the central penetrating wound were re-assessed at laparotomy to be of grade IV injury. These patients needed major liver resections to secure haemostasis.

It is our experience that patients with either grades I or II liver injuries (Table 3) can be successfully managed non-operatively provided no other indication for surgery is present and close monitoring is available. Non-operative management of these patients includes bed rest, nil orally, the nasogastric decompression when ileus is present, serial abdominal examinations, serial blood counts, and a repeat abdominal CT scan within the first 7 days. During the observation period, indications for laparotomy were: 1) Signs of an acute abdomen (4 patients in our series), 2) Haemodynamic instability or continued requirements for transfusion that cannot be accounted for by an extra-abdominal site of blood loss, 3) Progressive expansion of haematoma without encapsulation on CT scan, 4) Abdominal sepsis (8). The overall clinical picture of the patient, both at the initial evaluation and during the observation period, should dictate the need for laparotomy (3,15,16). In our series, none of the patients who were treated conservatively had laparotomy due to liver injuries. The 6 laparotomies were for gallbladder injuries in 2 cases, two nephrectomies and the other 2 patients were found to have only a 1cm haematoma over the anterior second part of duodenum (no perforation and no further intervention needed). All 6 patients recovered well.

Mortality of 8 out of 72 patients (11.1%) in the non-operative group was due to associated injuries to the thorax and head. This highlights the important fact that injuries to other vital organs contribute significantly to the final outcome of a patient with liver injuries.

It was unfortunate that only 38 of the 64 patients (59.4%) who survived in the non-operative group came for follow-up. Although this figure represents only about half of our patients, their follow-up CT scans with no long-term complications provide encouraging supporting data that non-operative approach is safe in patients with grade I or II liver injuries, provided that there is no associated significant haemoperitoneum and other abdominal visceral injuries that warrant immediate operative treatment.

Conclusion

Non-operative management of liver injuries due to blunt trauma, as detected by CT scan, is appropriate and successful in selected patients (grade I or II liver injuries) who are haemodynamically stable, without signs of an acute abdomen or massive intra-abdominal blood loss, and without other abdominal injury requiring laparotomy. The availability of frequent abdominal examinations by a surgeon and serial laboratory studies, as well as close monitoring in an intensive care unit is essential. Emergency laparotomy must be readily available should the need arise. Repeat ab dominal CT scans can verify resolution of the hepatic injury and should be performed before discharge or when clinically indicated. Long-term complications are rare (15-18), and none occurred in our group of patients seen in follow up. A prospective study is needed to determine the appropriate use of follow up CT scans in patients with blunt hepatic trauma.

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ORIGINAL ARTICLE

YOUNG COLORECTAL CANCER PATIENTS - A REVIEW OF 21 CASES

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ABSTRACT: All cases of primary colorectal carcinoma in patients below 30 years of age seen at the University Hospital Kuala Lumpur between 1990 - 1994 inclusive were reviewed. There were 21 cases seen during this period and 4 cases were less than 20 years of age (19%). The male:female ratio was 1:2. The majority of the patients were Chinese (about 14/21, 67%), with only 1 Indian patient (4%). 1 patient had a family history of colonic polyps. Most patients presented with advanced disease and the tumour was mucinous predominantly in histology. The overall 5 year survival was 25%. *(JUMMEC 1996 1(2): 49-52)*

KEYWORDS: carcinoma, colorectal, young

Introduction

Carcinoma of the colon and rectum is the second overall leading cause of death from cancer (after lung cancer) in Malaysia. In men, colorectal cancer ranks third, behind lung and prostate cancer in terms of new cancers diagnosed each year. It trails only lung cancer as a cause of death from cancer. In women, colorectal cancer ranks behind breast cancer in new cancer cases annually and behind lung cancer in causes of death by cancer (1). Clearly, colorectal cancer is primarily a disease of the older population. The median age for the diagnosis of colorectal cancer from 1982 through 1986 was 70 years (1).

Of all patients with colorectal cancer, approximately 0.6% to 5.4% are 30 years or younger (2-4), and approximately 3 to 10% are 40 years or younger (5-9)

This report represents our experience with patients suffering from colorectal cancer aged 30 years or younger through 1990-1994 at the University Hospital Kuala Lumpur, Malaysia.

Methods

The records of all patients aged 30 years or younger diagnosed or treated for colorectal cancer from1978 through 1992 at the University Hospital Kuala Lumpur were reviewed. Charts were reviewed for the following information: age, gender, race, site of tumour, presenting symptomatology, duration of symptomatology before diagnosis, histology, extension of tumour and nodal involvement, predisposing factors, treatment, and follow up.

Results

Age, gender & ethnic group distribution (Table 1)

Over the study period, 5 patients (24%) were 30 years old at diagnosis. Twelve patients (57%) were aged 20 through 29 years, and four patients (19%) were less than 20 years old. 13 of the 21 patients were female, and 8 (38%) were male. There were 14 Chinese (67%), 6 Malays (28%) and 1 Indian (5%).

Site of tumour (Table 2)

6 patients (29%) had their primary tumour located in the recto-sigmoid region, 4 (19%) in the left colon, one (5%) in the splenic flexure, two (10%) in the transverse colon, and five (24%) in the caecum. One patient had tumour too diffuse to detect a primary site at time of operation. One patient with a family history of polyps had his entire colon removed at age 14 years. He had 3 separate foci of tumour and was ultimately diagnosed with Turcot's syndrome.

Presenting symptoms

The most common presenting symptoms were pain in 12 patients (60%) and haematochezia or haemoccult-positive stool in 8 patients (40%). All

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patients with fresh blood per rectum had tumour localised to the rectum. Other common presenting symptoms included weight loss, nausea and vomiting, constipation, and diarrhoea.

Duration of symptoms

The average duration of symptoms was 2.6 months, with a range of 2 days to 9 months. In addition, one patient had ulcerative colitis, and diffuse carcinoma was found when the colon was removed for symptomatology of his disease.

Histology (Table 3)

The 21 patients had the following histology: 10 adenocarcinoma, 9 mucinous type adenocarcinoma, and 2 small cell carcinoma (undifferentiated).

Table 1

AGE DISTRIBUTION

Age	Number of patients
<20 years	4
20-29 years	12
30 years	5

Table 2

SITE OF TUMOUR

Site	Number of cases (%)
Rectosigmoid	6 (29%)
Left colon	4 (19%)
Splenic flexure	1 (5%)
Transverse colon	2 (10%)
Hepatic flexure	1 (5%)
Right colon	Nil
Caecum	5 (24%)
*Indeterminate	2 (10%)

*Ulcerative colitis & Turcot's syndrome

Table 3

HISTOLOGY OF COLORECTAL CANCER

Histology	Number of cases (%)
Mucinous adenocarcinoma	9 (43%)
Typical adenocarcinoma	10 (48%)
Small cell carcinoma	2 (10%)

Extension of tumour and nodal involvement

Sixteen of 21 patients (76%) had nodal involvement (Dukes' C) at the time of their initial surgery (7 mucinous, 7 typical, 1 small cell). Of the remaining 5 patients without nodal involvement, 1 patient had tumour of mixed mucinous and typical pathology that did not invade through the wall of the bowel *(Dukes'A)*. Another one had Dukes' *B* and tumour with mucinous pathology. The other 3 patients had Dukes' *B* typical adenocarcinoma that were poorly differentiated.

Predisposing condition

3 patients had conditions known to predispose to carcinoma. One patient diagnosed at age 29 had ulcerative colitis for 14 years. This patient was found to have diffuse carcinoma at laparotomy. He received palliative chemotherapy, but died within 1 year.

One patient diagnosed at age 14 years had a family history of polyposis. He was subsequently diagnosed to have Turcot's syndrome. He underwent a total colectomy with ileo-sigmoidostomy, chemotherapy and surveillance. He subsequently had an ileo-anal pull-through. He died 5 years later of glioblastoma multiforme, but was free of colon cancer.

One patient diagnosed at age 24 years had Gardner's *Syndrome*. She was found to have inoperable cancer at operation and died thereafter. Interestingly, a fourth patient diagnosed at age 11 had a Wilm's tumour resected at 5 months and received abdominal irradiation. He had a left colectomy, but was found to have diffuse tumour 3 years later. He died approximately 8 months after that.

Patient treatment and outcome

Of 21 patients, 14 (67%) underwent surgery with the intention of cure, five (24%) for palliation. No futher treatment was possible for two patients (10%). Twelve patients died within the follow-up period, of which eleven patients died of their colorectal carcinoma. Two

patients were alive with extensive cancer present at 18 months and 26 months, respectively, at last follow up. One patient had extensive disease at laparotomy, but was lost to follow up.

There were three *5-year* survivors (14%). One patient is alive at 14 years, another at 7 years. A third patient died at 5 years from a primary brain tumour. 2 patients who were less than 5 years from diagnosis were alive and disease-free at 30 months and 33 months, at last follow up. All the 3 patients had typical adenocarcinoma (1 Dukes' A & 2 Dukes' B).

Discussion

Approximately 4%-5% of the population can expect to develop colorectal carcinoma by age 75. Survival is 60%-70% for tumour without nodal involvement, depending upon the extent of tumour invasion of the bowel wall. Survival drops to 20%-50% when nodes are involved, and less than 5% when distant disease is encountered (12). Although this disease is relatively uncommon in the younger population, it is nonetheless well-recognised. A number of observations are important regarding colorectal carcinoma in the younger population.

Overall 5-year survival is worse in the younger population than in older population. Some reports suggest that for any given stage of disease, prognosis parallels that of the older population (5,9,13). However, more young patients present with advanced disease (5,6,8,9,11,13,14). Our series reflects the latter trend, as 16 of 21 patients (76%) presented with lymph node metastases. However, only 3 patients of 5 without lymph node metastases are long-term survivors.

It would seem that delayed presentation might account for advanced stage at presentation in young patients. Yet, in our series, many patients did not have inordinately long periods of symptoms. It is possible, as some assert, that a preponderance of tumours with mucinous histology account for more aggressive cancer presentation (6-8,11,15,16). Interestingly, one recent series reported a rather high 5-year survival rate of 57% for 55 patients with colorectal cancer aged 30 years and under. However, only 33% of the cancers were found to have mucinous or poorly differentiated histology (14).

This series would appear to be the exception, as other series of patients aged 40 years and under report an incidence of mucinous histology from 69% to 82% (3,4,11,17). The incidence in this series is 43% . Survival was exceedingly poor in all studies (7%-38%).

In our current studies, 3 patients were 5-year survivors. All had typical adenocarcinoma with negative nodes. None of the mucinous type survived the period. This would suggest a trend toward a poor outcome for those patients with mucinous histology, in our institution's experience.

Despite the relatively unusual occurrence of colorectal cancer in young patients, relatively few have predisposing causes (4,8,10,15,18). Only 3 patients in our series, had conditions known to predispose to the development of colorectal cancer.

Pain and blood per rectum are the most common symptoms manifested in patients with colorectal cancer in our experience. This is reflected in other series as well (5,11,18). In addition, in our patients the preponderance of tumours were in the rectosigmoid region, which is again consistent with other reports (2,7,8,14).

The surgeon should entertain the possibility of colorectal carcinoma in any young patient who presents with abdominal pain, haematochezia, haemoccult-positive stools, weight loss, or development of persistent constipation, diarrhoea, nausea or vomiting. Young people have a predilection for mucinous type pathology that appears to be quite aggresive. Thus, it is imperative that symptoms in young people be investigated promptly in order to detect these tumours at an early stage. Colorectal cancer at an early stage may still be successfully treated in young people.

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CASE REPORT

CULTURE-CONFIRMED *CHLAMYDIA PNEUMONIAE* INFECTION

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ABSTRACT: **A strain of *Chlamydia pneumoniae* was isolated from the throat swab of a 45 year-old female patient with tracheobronchitis who responded well to doxycycline therapy. The isolate was confirmed by fluorescent antibody staining and by polymerase chain reaction. As there are no distinct clinical features to indicate *C pneumoniae* respiratory infections, laboratory confirmation is required for definitive diagnosis.** *(JUMMEC 1996 1(2): 52-56)*

KEY WORDS: *C. pneumoniae*, **culture, tracheobronchitis**

Introduction

Chlamydia pneumoniae is a recently recognized cause of human respiratory infections. Clinical manifestations associated with this organism resemble those caused by *Mycoplasma pneumoniae* and include pharyngitis, sinusitis, bronchitis, pneumonia and brochial asthma (1,2). Laboratory confirmation is difficult as the isolation of the bacteria requires cell culture. Non-culture methods using enzyme immunoassay (EIA), direct fluorescent antibody staining (DFA) and DNA probes which have been commonly used for the detection of *C. trachomatis* have not been adequately evaluated for *C. pneumoniae*. Polymerase chain reaction (PCR) is increasingly being used to determine the presence of the organism in clinical specimens but is still a tool largely confined to the reference laboratory. Most investigations have relied on serologic diagnosis.

In Malaysia, serodiagnosis has associated *C. pneumoniae* with 13-15% of community-acquired acute lower respiratory tract infections in adult patients (3,4) but only 1.2% among infants and children under 3 years old (5). This paper reports a culture-confirmed case of tracheobronchitis in a local patient.

Case Study

A 45 year-old female travel consultant presented with a month's history of productive cough and wheezing which had not responded well to treatment with cephalexin and cotrimoxazole. She did not smoke cigarettes. On examination, no clinical abnormalities were found apart from an occasional wheeze. Her peak flow rate was 280 L/min. She was diagnosed as a case of tracheobronchitis.

A throat swab taken from the patient was transported to the laboratory in SPG (sucrose-phosphate glutamic acid) chlamydia transport medium kept ice-cold during transportation and cultured on the day of collection. After 3 days of incubation, typical intracytoplasmic inclusions were seen in the cell monolayer *(Figure 1)*. Two PCRs amplifying different targets in the *C. pneumoniae* genome, were carried out on the infected culture. The throat swab was also tested directly by PCR without prior culture. The respective PCR products visualized by gel electrophoresis confirmed the presence of *C. pneumoniae* in the patient's specimen *(Figure 2 and 3)*.

As the patient was allergic to macrolides, she was treated with doxycycline 100mg daily for 10 days. At the end of treatment, her symptoms had resolved and her peak flow rate had increased to 370 L/min. A repeat throat swab taken 16 days after treatment was culture and PCR negative and remained culture-negative after 2 blind passes.

Material and Methods

Chlamydial culture

Isolation of chlamydiae was done in HEp-2 cells kindly provided by Professor Akira Matsumoto of Kawasaki Medical School, Japan. The throat swab was vortexed in the SPG transport medium and the resulting suspension was sonicated at output 80 (Branson Sonifier 250) for 4 minutes. Two aliquots of 200 µL of the specimen were centrifuged at room temperature for 45 min at 2000 x g onto HEp-2 cells grown on 13mm coverslips in shell vials. The inocula were then replaced by Eagle's

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Figure 1: Intracytoplasmic inclusions of *Chlamydia pneumoniae* (indicated by arrows) cultured in HEp-2 cells and stained with FITC-conjugated monoclonal antibody to *C pneumoniae* major outer membrane protein.

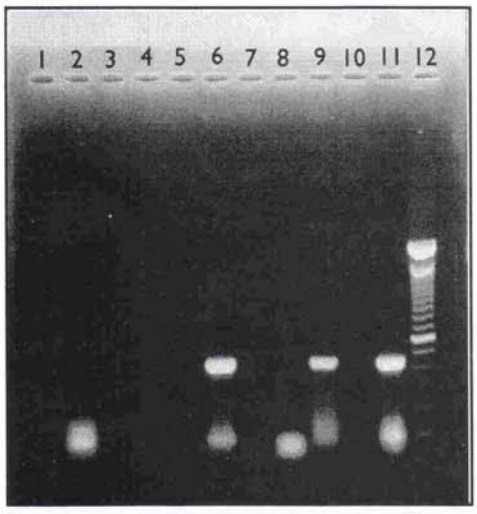

Figure 2: *C pneumoniae* PCR products (463 bp amplified with CpnA-CpnB primer set) in 1.2% agarose gel electrophoresis. Lanes 1: negative control, lanes 3,4,5,7:blanks, lane 11:positive control, lanes 8,9,10:3 passes of a control *C pneumoniae* culture, lane 2:patient's throat swab, lane 6:patient's throat swab culture.

Figure 3 : *C pneumoniae* PCR products (499 bp amplified with 53.1-53.2 primer set) in 1.2% agaros gel electrophoresis. Lanes 1-4,6,8,10,12: negativ control and blanks, lane 9:positive control, lan 5:patient's throat swab culture diluted 1:5, lan 7:patient's throat swab *culture neat.*

MEM (Gibco Laboratories, Chicago, USA) supplemented by 10% fetal calf serum, 1000 µg/ml gentamicin, 0.2 ug/ml streptomycin, 250 µg/ml amphotericin B and 1 µg/ml cycloheximide and the cultures incubated in 5% CO_2 at 35° C for 3 days. The infected monolayers were then fixed with acetone and stained with FITC-conjugated monoclonal antibody to *C pneumoniae* major outer membrane protein (MOMP) (DAKO A/S Denmark). *C. pneumoniae* appeared as apple-green intracytoplasmic inclusion bodies under the epifluorescence microscope.

Chlamydial PCR

The 2 primer sets used for the PCR were CpnA-CpnB (6) which amplified a target on the *C pneumoniae* MOMP gene and 53.1-53.2 (7) which amplified the gene encording a *C pneumoniae*-specific immunodominant protein. CpnA-CpnB was a gift from Dr Charlotte Gaydos, The Johns Hopkins University, USA. The patient's specimen was incubated with proteinase K (100 µg/ml) and Nonidet P40/Tween 20 (0.5% v/v) at 55°C for 1 h and then boiled for 5 min. Five µl of this sample was used for the PCR in a 50 µl reaction volume containing PCR buffer (10 mM Tris pH 8.3, 50 mM EDTA), 200 µM concentrations of each deoxynucleotide triphosphate, 2.5 mM $MgCl_2$, 0.5 µM concentrations of each primer and 1 unit of Taq DNA polymerase (Promega, U.K.). Amplifications were carried out in a Perkin-Elmer 480 thermal cycler with 35 cycles of 94° C x 1 min, 55° C x 1 min and 72° C x 2 min. The positive control used was a suspension of *C. pneumoniae* elementary bodies processed as for the patient's specimen while the negative control consisted of the PCR reaction mixture without DNA. At the completion of amplification, PCR products were analyzed by 1.2% agarose gel electrophoresis. The presence of *C. pneumoniae* was indicated by a 463 bp band in the gel with CpnA-CpnB primers and a 499 bp band with the 53.1-53.2 primer set. The specificity of the bands were not determined by hybridization with specific probes but the use of 2 PCRs amplifying different targets in the *C. pneumoniae* genome helped to reduce the possibility of non-specific amplification.

Discussion

The routine diagnosis of *C. pneumoniae* infections by serology is not entirely satisfactory as serologic results are difficult to interpret. Antibody response may be delayed or absent altogether, especially in children. Cross-reactions between *C. trachomatis* and *C.. pneumoniae* are not infrequent and diagnostic titres are often found in healthy asymptomatic persons (8).

The isolation of *C. pneumoniae* is confirmatory but this organism has been reported to grow poorly in tissue culture, often requiring multiple passages for positive isolation from clinical specimens. The inclusion bodies produced tend to be smaller and fewer than for other chlamydial species (1). Many laboratories experienced in *C. trachomatis* culture had had difficulty isolating *C. pneumoniae* and even in the best laboratories, the sensitivity of culture compared to non-culture methods is less than 90% (8). Hence, false culture-negatives are common. On the other hand, a positive culture does not always indicate disease as there is evidence that the organism can be carried in the apparently healthy respiratory tract. Isolation rates of 2% and 5% respectively have been found among asymptomatic adults and children (8) and in a Finnish outbreak, it was noted that only 1 in 10 infections resulted in clinical disease (9).

PCR is the most promising non-culture method for the diagnosis of *C .pneumoniae* infection although it can also give false negatives due to the presence of polymerase inhibitors and nucleases in clinical specimens as well as false positives due to amplicon contamination in the laboratory. Compared to culture and serology, it can certainly provide a more rapid diagnosis for the administration of appropriate antibiotic treatment.

In the presence of clinical features which are known to be associated with *C. pneumoniae* infection, especially the prolonged symptoms which did not respond well to treatment with antibiotics not totally effective against chlamydia but disappeared on treatment with doxycycline, it is most likely that the *C .pneumoniae* isolated from the patient was the cause of her illness. *Mycoplasma pneumoniae* and *Legionella pneumophila* can also cause similar clinical presentations and co-infection with *C. pneumoniae* is possible. A multiplex PCR for the detection of all 3 pathogens would greatly facilitate the diagnosis of respiratory infections caused by these organisms.

Most studies on *C. pneumoniae* infection in Malaysia and many other parts of the world have not had culture-proven cases. This report documents the role of this organism as a cause of airway infection in Malaysia.

Acknowledgments

The authors are grateful to Professor Akira Matsumoto of Kawasaki Medical School, Japan and Dr Charlotte Gaydos of The Johns Hopkins University, USA for their generous gifts of HEp-2 cells and primers for the *C .pneumoniae* culture and PCR.

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ABSTRACTS

MICROVASCULAR CHANGES IN LIVER AFTER ISCHEMIA-REPERFUSION INJURY: PROTECTION WITH MISOPROSTOL

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Morphological changes in the hepatic microvasculature were studied in experimentally induced ischemia-reperfusion injury in the rat using a vascular casting technique. Partial hepatic ischemia was induced for 90 min followed by 24 hr of reperfusion. Microvascular casting was performed after 24 hr reperfusion by either intraarterial or intravenous infusion of acrylic resin (Mercox). After corrosion of the tissue, the cast was examined by scanning electron microscopy. Casts of normal livers showed good patency with no evidence of unfilled areas. The mean diameter of sinusoids was 14±3 mm with those in zone 1 slightly smaller than those in zone 3. Liver casts form rats subjected to ischemia and reperfusion resulted in gross disruption of normal architecture. The common characteristics seen in both prograde and retrograde casts were clusters of closed sinusoids around zones 2 and 3 of the liver acini, which resulted in cavities of various sizes. Varicosities were observed in some areas. The mean diameter of sinusoids in areas of patent microvascular structure (10±2 mm) was significantly smaller compared to those in normal livers ($p<0.001$). Misoprostol given at 1 min before reperfusion markedly reduced the microvascular injury. The hepatic microvasculature was generally intact with mild focal unfilled areas. The majority of the sinusoids were of normal size and no clusters of blind ending sinusoids were detected. The present study shows that hepatic ischemia-reperfusion results in extensive microvascular injury in the liver. The protective effects of misoprostol against this injury may occur at the vascular level.

(Dig Dis and Sci 1994; 39:1683-1690)

ACETAMINOPHEN-INDUCED MICROVASCULAR INJURY IN THE RAT LIVER: PROTECTION WITH MISOPROSTOL

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Studies into the mechanism of acetaminophen (APAP)-induced hepatotoxicity have focused mainly at the hepatocellular level. This study aimed to investigate the effect of APAP on the hepatic microvasculature using a vascular casting technique. APAP was administered at a dose of 650 mg/kg body weight (intraperitoneally) to fasted male Long Evans rats. Microvascular casting was performed at various points after drug administration. Liver casts from control rats showed good patency with normal hepatic microvasculature. Thirty-six hours after overdose with APAP, liver casts showed rounded centrilobular cavities of various sizes, representing regions in which cast-filled sinusoids were absent with relatively normal microvasculature within periportal regions. Evidence of microvascular injury occurred as early as 5 hours after APAP overdose. This injury consisted of changes to centrilobular sinusoids including areas of incomplete filling and dilated centrilobular sinusoids. Misoprostol (a prostaglandin E_1 analog) treatment (6 x 25 mg/kg) given before and after APAP administration markedly reduced the extent of microvascular injury with only small focal unfilled areas in the casts and a generally intact microvasculature. In conclusion, this study shows that overdosage with APAP resulted in an extensive, characteristic pattern of hepatic microvascular injury in the centrilobular region. The results also suggest that microvascular injury is an early event in the pathogenesis of APAP hepatotoxicity. Misoprostol was found to protect against injury occurring at the microvascular level.

(Hepatology 1995;22:1776-1781)

EPSTEIN-BARR VIRUS INFECTION IN CARCINOMA OF THE SALIVARY GLAND

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Undifferentiated carcinoma of the parotid gland contains clonal Epstein-Barr virus episomes without ladder arrays of restriction enzyme fragments representing virion DNA. Analysis of Epstein-Barr virus transcription in situ in parotid carcinoma specimens revealed that the EBER RNAS, latent membrane protein MRNA, and the BamHI-A rightward reading frame, BARFO, are expressed in the malignant epithelia] cells.

(J Virol 1991; 65:249-252)

HUMAN INFECTION WITH *DIROFILARIA REPENS* IN MALAYSIA

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Human dirofilariasis is a rare infection in Malaysia. Thus far, only two human cases have been reported viz. *Dirofilaria immitis* and D. *(Nochtiella) repens* and in both instances, adult worms were recovered from infected patients. The two cases reported in the present study, one from Melaka and the other from Penang, were diagnosed histologically. Based on the diagnostic criteria for identifying *Dirofilaria* in tissue sections, the parasites were identified as *D. (Nochtiella) repens*.

(J Helminthology 1996; 70:249-252)

COMPARISON OF TWO MONOCLONAL ANTIBODY KITS WITH CELL CULTURE ISOLATION IN THE DETECTION OF RESPIRATORY VIRUS ANTIGENS

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Two different preparations of monoclonal antibodies developed against respiratory viruses have been evaluated by the immunofluorescence antibody technique. The Chemicon monoclonal antibodies were found to be more efficient at picking up positive specimens with a high sensitivity and specificity than Imagen monoclonal antibodies. However, the overall concordance rate of the monoclonal antibodies was 92.3%-100%. Generally, when compared with cell culture isolation, the immunofiourescence antibody technique was found to be more sensitive. The high quality of the Chemicon monoclonal antibodies contribute to their value in providing definitive diagnosis, within a few hours of specimen collection, thus allowing early management of patients, their contacts and control of hospital infection.

(Malaysian J Pathol 1996; 18(1):27 - 30)

AN IMMUNOHISTOCHEMICAL METHOD FOR THE DIAGNOSIS OF MELIOIDOSIS

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In order to assess the usefulness of immunohistochemistry in the diagnosis of melioidosis, an infection by *Burkholderia pseudomallei*, polyclonal antibodies were applied to tissues from known cases of melioidosis and to other infected tissues. Formalin-fixed, paraffin-embedded tissues were stained by a modified immunoperoxidase technique. In autopsy tissues with inflammatory lesions of melioidosis, the cytoplasm of phago-cytes and intact bacilli, both intra- and extraceliular, were stained very strongly positive. Relatively more focal positive staining was observed in some but not all surgical biopsies from proven cases of melioidosis. In granulomas staining was mainly found in the central necrotic areas, with little staining of peripheral phagocytes. All control materials stained negative. fmmunohistochemistry appears to be a useful diagnostic tool in melioidosis.

(Pathology 1996; 28:188-191)

MITOCHONDRIAL ABNORMALITIES IN OCULOPHARYNGEAL MUSCULAR DYSTROPHY

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This report describes a 56-yr-old man with a dominantly inherited disorder affecting four generations and characterized by bilateral ptosis and dysphagia. Muscle biopsy showed only minor light microscopic abnormalities but electron microscopy revealed fibres containing paraerystailine mitochondrial inclusions. Southern analysis of mitochondrial DNA obtained from muscle did not reveal mitochondrial gene deletions. An extensive search eventually identified the characteristic intranuclear filaments of oculopharyngeal muscular dystrophy (OPMD). Abnormal mitochondria are nonspecific epiphenomena in OPMD but a potential source of confusion with a late-onset mitochondrial cytopathy. This case further emphasizes the necessity for a diligent search for the diagnostic intranuclear filaments when oculopharyngeal muscular dystrophy is suspected clinically.

(Neuromusc Disord. 1996; 6(3):163-166)

INTRACRANIAL GERMINOMA METASTASIZING VIA AVENTRICULO-PERITONEAL SHUNT

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Primary germinomas of the central nervous system carry a good prognosis because of their radiosensitivity. Recurrences are rare and extraneural metastases are even more unusual One of the possible routes of extraneural spread is via ventriculo-peritoneal shunts which may be required to reduce intracranialpressure. One such case of germinoma metastasizing via a ventriculo-peritoneal shunt is reported. Patients with intracranial germinomas and ventriculo-peritoneal shunts should have close surveillance of their abdomens and may require systemic chemotherapy.

(Singapore Med J 1996; 37:441-442)

CLONAL PROLIFERATIONS OF CELLS INFECTED WITH EPSTEIN-BARR VIRUS IN PREINVASIVE LESIONS RELATED TO NASOPHARYNGEAL CARCINOMA

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Background. The Epstein-Barr virus (EBV) is consistently detected in patients with nasopharyngeal carcinoma. To determine whether EBV infection is an early, initiating event in the development of this malignant tumor, we screened nasopharyngeal-biopsy samples, most of which were archival, for preinvasive lesions, including dysplasia and carcinoma in situ. Preinvasive lesions were found in 11 samples, which were tested for the presence of EBV.

Methods. EBV infection was detected with in situ hybridization for EBV-encoded RNAs (EBERS) and by immunohistochemical staining for latent membrane protein 1 (LMP-1). The larger samples were also tested for the EBV genome with the use of Southern blotting. The expression of specific EBV RNAs was determined by the amplification of complementary DNA with the polymerase chain reaction.

Results. Evidence of EBV infection was detected in all 11 tissue samples with dysplasia or carcinoma in situ. EBERs were identified in all eight samples tested, and LMP-1 was detected in all six of the tested samples. Six of the seven samples tested for the EBV termini con-tained clonal EBV DNA. Transcription of the latent EBV gene products, EBV nuclear antigen 1, LMP-1, LMP-2A, and the BamHI-A fragment, was detected in most of the samples. Viral proteins characteristic of lytic lesions were not detected.

Conclusions. Preinvasive lesions of the nasopharynx are infected with EBV. The EBV DNA is clonal, indicating that the lesions represent a focal cellular growth that arose from a single EBV-infected cell and that EBV infection is an early, possibly initiating event in the development of nasopharyngeal carcinoma. Preinvasive lesions contain EBV RNAs that are characteristic of latent infection but not the viral proteins that are characteristic of lytic infection. The detection of the EBV-transforming gene, LMP-1, in all the neoplastic cells suggests that its expression is essential for preinvasive epithelial proliferations associated with nasopharyngeal carcinoma.

(N Engl J Med 1995; 333:693-698)

CUTANEOUS ADNEXAL NEOPLASMS IN BIOPSY SPECIMENS PROCESSED IN THE DEPARTMENT OF PATHOLOGY, UNIVERSITY OF MALAYA

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A review of consecutive biopsies of adnexal tumours from 112 patients, received by the Department of Pathology, University of Malaya, over a 13-year period was undertaken. The age range of the patients was from I to 84 years, with a mean of 29.8 years. Thirty-three (32%) patients were under 20 years of age. There were 68 females with a male to female ratio of 1.0:1.5. In 105 cases (93.7%), the neoplasm was solitary. The tumour measured less than 2 cm in the largest dimension in 103 cases (92%). The common sites of occurrence were the head and neck region (59%) and extremities (25%). Neoplasms of hairfollicle origin accountedfor 63.4% (71 cases) of all lesions. Intra-tumour deposition of amyloid was noted in one of the 14 cases of trichoepithelioma.

(Ann Acad Med Singapore 1996; 25:522-5)

AN IMMUNOHISTOCHEMICAL STUDY OF NEUROFIBRILLARY TANGLE FORMATION IN POST-ENCEPHALITIC PARKINSONISM

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Neurofibrillary tangle (NFT) formation is a feature of postencephalitic Parkinsonism (PEP) and Alzheimer's disease (AD). Tangle formation has been compared immunohistochemically in these 2 conditions. Staining patterns for tau protein, ubiquitin and b/A4 arnyloid protein were studied in frontal lobe, hippocarnpus, and midbrain in 2 classical cases of PEP, 2 cases of AD and 2 controls matched for age and sex. NFI's were present in all cases, but with varying frequency: all tangles were tau-positive and many were ubiquitinated. In the frontal cortex and hippocampus, irrespective of the case category, tangle formation was associated with P/A4 amyloid deposition. A similar association was present in the 2 AD cases in the midbrain. However, in PEP tangle formation in the midbrain was not associated with adjacent b/A4 arnyloid deposition. This finding raises the possibility that the pathogenetic mechanism of tangle formation in PEP is different from that of AD, although the final cellular morphological expression of abnormality in both conditions is similar.

(Clinical Neuropathology 1996; 5(I): (22-25)

MICROCALCIFICATION CLUSTERING PARAMETERS IN BREAST DISEASE: A MORPHOMETRIC ANALYSIS OF RADIOGRAPHS OF EXCISION SPECIMENS

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X-ray microradiography of surgically excised breast specimens offers the possibility of morphological characterization of calcifications. When combined with digital imaging techniques there exists added potential for obtaining valuable basic quantitative morphometric information regarding differences between microcalcifications in tissues exhibiting evidence of fibrocystic change, benign and malignant tumours. A total of 157 excised breast specimens from 84 patients were microradiographed using a Softex Super Soft X-ray unit and Kodak AA high resolution industrial film. A Quantimet 57OC image analysis system was used to digitize and analyse the microradiographs. Of the 157 microradiographs, 51 (from 30 patients) revealed microcalcification clusters. The existence of significant differences between the three identified categories of tissue were indicated by clustering parameters. These included the number of particles per cluster, area of clusters, maximum distance to nearest neighbour, and geometric mean distance to nearest neighbour. The distribution pattern index (DPI), another of the clustering parameters used in this study, has been observed to be a particularly powerful discriminator. The value for fibrocystic change was found to be significantly smaller (0.514) than that for benign tumour (0.796) whilst that for benign tumour was observed to be significantly larger than that for malignant tumour (0.604) at a p-value of less than 0.05 (Kruskal-Wallis one-way analysis of variance).

(The British J of Radiology, 1996; 69: 326-334)

DISCRIMINANT ANALYSIS OF NORMAL AND MALIGNANT BREAST TISSUE BASED UPON INAA INVESTIGATION OF ELEMENTAL CONCENTRATION

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Discriminant analysis of six trace element concentrations measured by instrumental neutron activation analysis (INAA) in 26 paired-samples of malignant and histologically normal human breast tissues shows the technique to be a potentially valuable clinical tool for making malignant-normal classification. Nonparametric discriminant analysis is performed for the data obtained. Linear and quadratic discriminant analyses are also carried out for comparison. For this data set a formal analysis shows that the elements which may be useful in distinguishing between malignant and normal tissues are Ca, Rb and Br, providing correct classification for 24 out of 26 normal samples and 22 out of 26 malignant samples

(Appl Radiat Isot 1997; 48(1):105-109, 1997)

APPLICATIONS OF XRF, NAA AND LOW-kV RADIOGRAPHIC TECHNIQUES IN THE STUDY OF BODY COMPOSITION AND DISEASED TISSUE

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Members of this group have responded to a number of challenging health issues by attempting to devise sensitive XRF, NAA and low-kv radiographic measurement systems for both *in vivo and in vitro* applications. These studies are generally either of toxicological importance, examine potential for diagnosing the presence of disease, or offer effective means for monitoring potentially harmful side-efrects of therapy. Particular examples include the *in vivo* XRF investigation of human skeletal uptake of Pb in working and living environments, *in vivo* XRF monitoring of elevated levels of Fe in skin (indicating the presence of an undesirable side-effect of the treatment of thalassaemia), *in vivo* NAA monitoring of elevated levels of AI in bone (indicating an undesirable side-effect of the treatment of chronic renal failure) and *in vitro* characterization, by means of low-kv imaging, of a range of calcification parameters in heathy and diseased breast tissue. The latter investigation has been conducted in association with an *in vitro NAA* study of concentrations of trace elements in the same types of tissue. Figures of merit for the various measurement systems have been obtained in terms of minimum detectable levels and concentrations (MDL's and MDC'S) and where applicable, image related parameters.

(Radiat Phy Chem 1996; 47(5):745-749)

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