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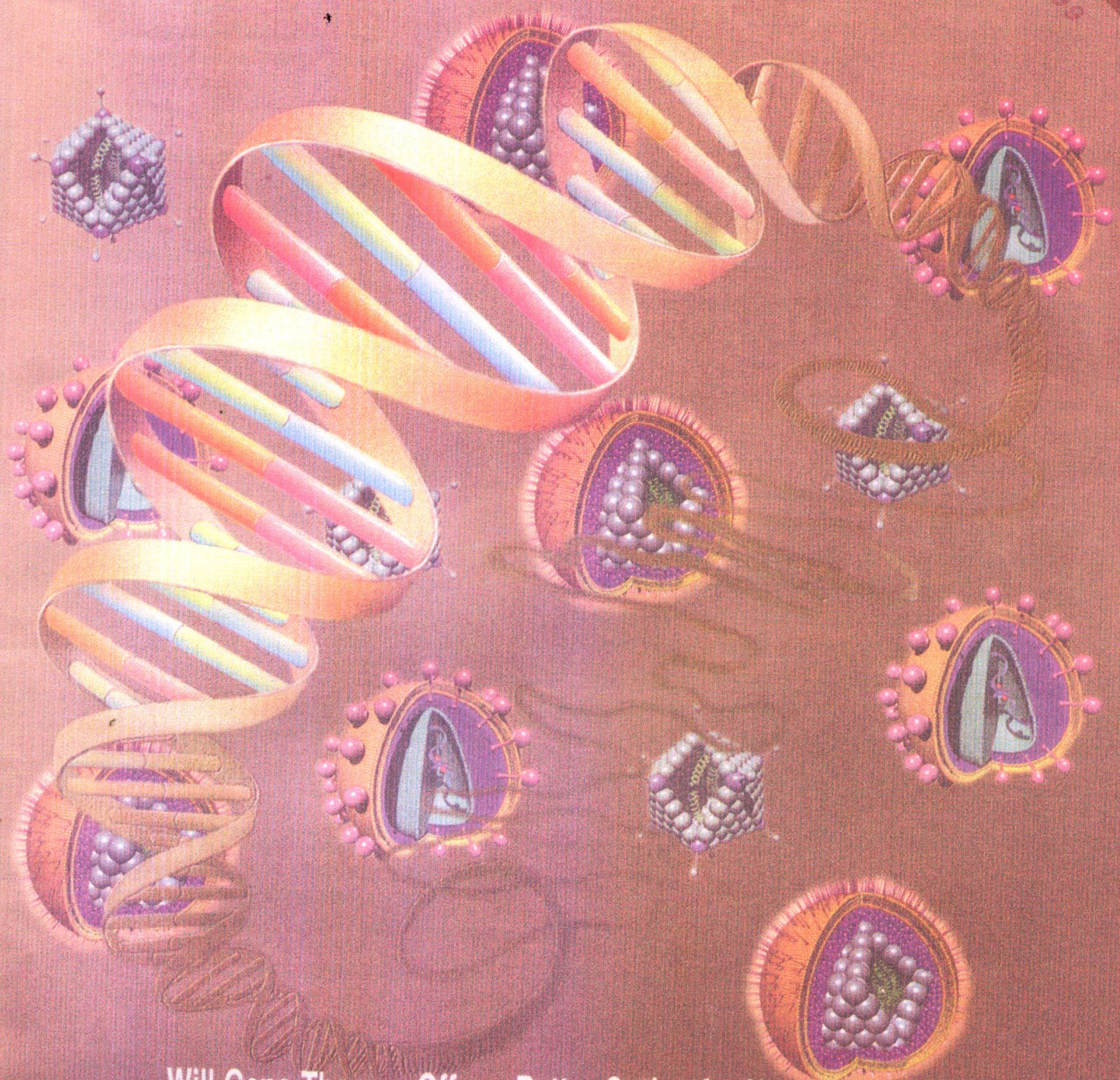
Gene Therapy



Will Gene Therapy Offer a Better Option for Neurological Disorders

Gene Therapy: A New Horizon for Medical Science

MEDIQUIZ

ORION LABORATORIES LIMITED

Speed and Power

Maprocin

Ciprofloxacin (Tablet & Injection)

Acts

Fast and Decisively

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Cover illustration: A model of plasmid DNA molecule on a background of viral gene therapy vectors

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EDITORIAL

This is our second issue of "The Orion", medical journal published by the Medical Services Department of Orion Laboratories Ltd. We have received overwhelming support and appreciation as well as valuable comments and suggestions regarding our inaugural issue of "The Orion". We offer to you our deepest gratitude.

We hope that the topic "Antibiotic Resistance" which was highlighted in our previous issue would be helpful in generating awareness among our professional colleagues about the impending global danger of antibiotic resistance. Considering our mission to update and upgrade our knowledge about the latest development in medical science, in this issue we are focusing on "Gene Therapy".

Gene therapy has tremendous prospect for genetic as well as non-genetic diseases where conventional medical therapy could not provide effective treatment. Gene therapy is a new horizon for medical science. The application of gene therapy is of particular importance since current traditional therapy is inadequate for many diseases. The significant development in the understanding of pathogenesis of some burning problems of various clinical disorders during the past few years form the basis of development of gene therapy. In this respect scientists are devoting themselves wholeheartedly in the pursuit of discovering new clinical approaches. We, the doctors community, are eagerly waiting to harvest the fruits of their endeavour to alleviate the sufferings of mankind.

We wish our esteemed readers a happy New Year and the Holy Eid greetings.

Dr ATM Azizur Rahman
Manager
Medical Services Department

MSD news

A VITAL ACTIVITY OF MSD IS THE ONGOING CLINICAL TRIALS TAKING PLACE IN THE SURGERY UNIT-IA OF BANGABANDHU SHEIKH MUJIB MEDICAL UNIVERSITY (BSMMU), DHAKA AND MEDICINE UNIT-I OF DHAKA MEDICAL COLLEGE HOSPITAL (DMCH), DHAKA.

Efficacy Trial in BSMMU

An efficacy trial on MAPROCIN™ (ciprofloxacin) and NIDAZYL™ (metronidazole) both parenteral and oral forms has been started in Surgery Unit-IA, Bangabandhu Sheikh Mujib Medical University, Dhaka under the direct supervision of Prof. ANM Atai Rabbi, FCPS, FICS, Chairman and Head of the Department of General Surgery. The objective of the trial is to scientifically assess the efficacy of MAPROCIN™ and NIDAZYL™ both parenteral and oral forms among post-operative patients.

Clinical Trial in DMCH

Under the direct supervision of Prof. Firdous Ara J. Janan, FCPS, MD, FRCP, Professor and Head of the Department of Medicine, a clinical trial is now in progress in Medicine Unit-I. The purpose of the trial is to evaluate the efficacy of **triple therapy** using ORIXYL™ (amoxycillin), NIDAZYL™ (metronidazole) and ORTAC™ (ranitidine) in the eradication of *Helicobacter pylori*, the much discussed pathogen responsible for peptic ulcer disease. Approximately 60 (sixty) patients will receive this regimen after undergoing endoscopic examination and Rapid Urease Test. Simultaneously, a second trial is also going on to assess the efficacy of ORTAC™ (ranitidine) on healing of peptic ulcer employing the same number of patients.

The success of these trials will help establish the quality and merit of Orion products.

MediQuiz winners

We are happy to announce that there has been an overwhelming response to the MediQuiz in the inaugural issue. A lottery was held to draw out the lucky winners among the participants who have given all correct answers. We thank the participants and congratulate the winners:

The winners of the MediQuiz, inaugural issue are:

1. Dr MMR Chowdhury, MBBS, MRCP (Part I, UK)
2. Dr Nazmul Huda Sethu, MBBS
3. Dr Shalauddin Kaiser, MBBS
4. Dr Farida Yasmin, MBBS, FCPS (Gynae), DGO, MCPS
5. Dr SM Mustafa Zaman, MBBS, DTCD
6. Dr Faisal Been Kashem, MBBS
7. Dr MA Gafur, MBBS, FCPS (Surgery)
8. Dr Kishwar Sultana, MBBS
9. Dr Md Daulatuzzaman, MBBS, MCPS, DLO
10. Dr M Rahman Chowdhury, MBBS

The winners will be contacted individually and awarded a full set of Cecil's Textbook of Medicine latest edition each.

WILL gene therapy offer a better option for neurological disorders

Dr Mahbubur Rahman, Dr ATM Azizur Rahman

In the past few years (10-15 years), researchers are working hard in different parts of the world to apply gene therapy - i.e. modification of the function or structure of gene for cure and control of disease. It is usually carried out by introducing a functional gene to replace or supplement the activity of a resident defective gene. Gene therapy has tremendous prospects for genetic as well as non-genetic diseases where conventional medical therapy (pharmacological, surgical or dietary) could not provide effective treatment. Normal human nerve cell (neuron) carry 2 sets of 23 chromosomes, one set inherited from the father and the other from the mother. The DNA in each chromosome carries thousands of genes and each gene contains codes for a single protein. Genetic nervous diseases arise when one or more related gene becomes altered such that a neuron produces a different protein than that needed for normal cell metabolism. Such an altered gene or genes (mutated genes) may produce disease. Genetic alterations in nervous system are not only inherited but also can be acquired during the life of a person.

The therapeutic (correcting) gene of interest is introduced to neurons (recipient cell) through viral or non-viral system (Figure 1). The exogenous gene can be delivered to the patients by ex-vivo or in-vivo system. In commonly used ex-vivo delivery, neurons from the patient are removed for introduction of therapeutic or healthy gene into these neurons that are then returned to the patient. In in-vivo delivery, the normal gene is delivered directly to the target neurons of the affected organ in an intact individual, thus avoiding cell transplant.

Unlike other tissues of the body, neurons (nerve cells) has certain unique properties that make them vulnerable:

- Neurons in central nervous system are unable to divide.
- Neurons that are lost are gone for good. Injured nerve tissues of the brain and spinal cord cannot be expected to repair itself.
- Central nervous system is prone to be damaged by sudden crisis such as stroke, epilepsy or head injury etc.

In the past few years, researchers have learned a great deal about how neurons die after a sudden medical insult such as stroke, seizure or head injury, as well as during progressive diseases such as Parkinson's or Alzheimer's. Some attempts to take advantage of these recent discoveries suggest that administering certain drugs may protect threatened neurons or even that lowering the temperature of the brain can avert the death of fragile cells during a neurological crisis. What is more, new knowledge about how neurons succumb to various diseases has raised the exciting possibility of protecting these cells by modifying their genes.

Genes instruct cells to make specific proteins, such as the enzymes that catalyze various chemical reactions. Nerve cells, for example, produce enzymes that synthesize neurotransmitters - substances that carry chemical signals across the tiny gaps (synaptic spaces) between one neuron and another. Gene therapy targeted to failing neurons could potentially provide them with a

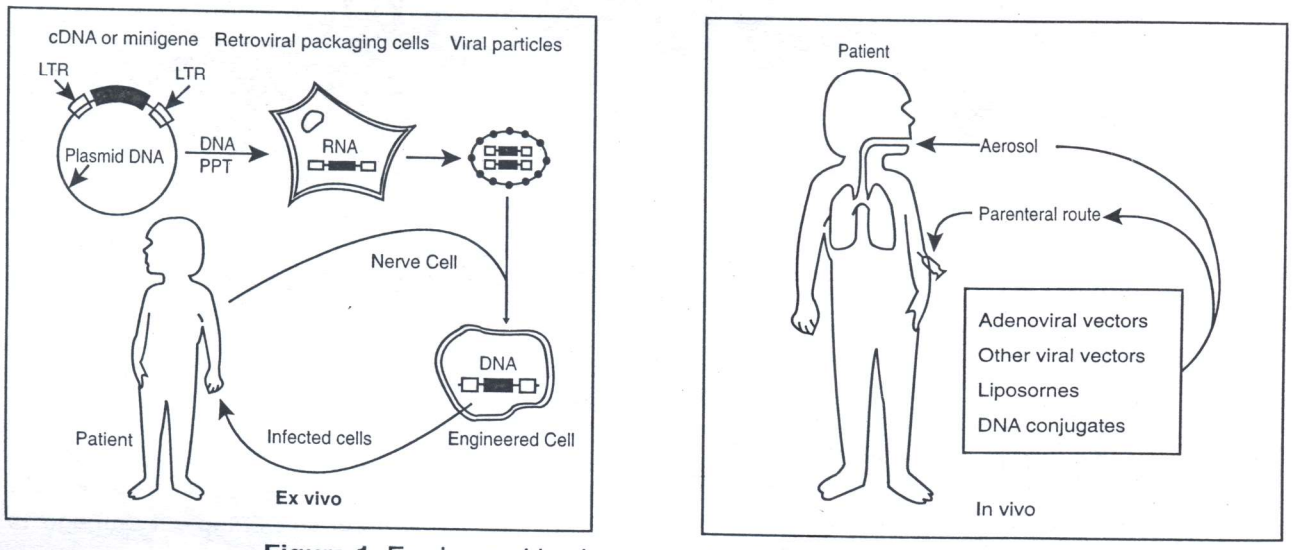


Figure 1. Ex vivo and in vivo strategies for somatic gene therapy.

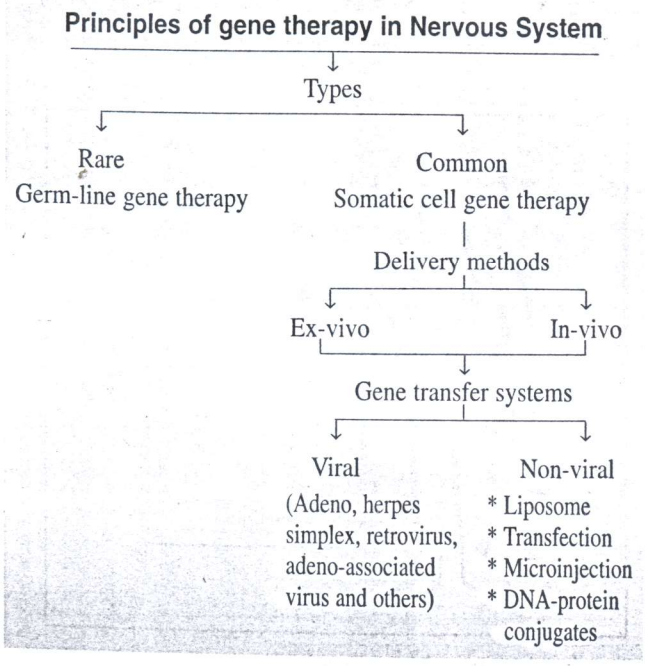
Dr Mahbubur Rahman, MBBS, MSc, PhD
Dr ATM Azizur Rahman, MBBS, Manager (MSD)

gene specifying a protein that is able to shield these cells from whatever threat may loom.

The application of gene therapy to neurologic diseases is of particular importance since the current traditional therapy is inadequate for so many of these diseases. Disorders such as Parkinson's disease or motor neuron diseases (amyotrophic lateral sclerosis) progressively rob normal movement and control of the body. Damage in cerebral cortex or spinal cord can create equal misery in just an instant. The neurodegenerative disease Alzheimer's disease attacks the very essence of one's personality as it destroys the mind. The significant developments in the understanding of pathogenesis of some neurologic disorders during the past few years form the basis of development of gene therapy for these diseases.

Parkinson's disease

Parkinson's disease (PD) is characterized by progressive loss (death) of dopaminergic (nigral) neurons in substantia nigra (projecting into the striatum) that secrete the neurotransmitter dopamine. Substantia nigra helps to regulate motor control, and its destruction makes it hard for a person to initiate movements or execute complex coordinated motion with the appearance of classic Parkinsonian tremor. Current treatments ameliorate symptoms and signs and temporarily improve quality of life accompanied by adverse motor and mental effects. No treatments lessen the progressive pace of nigrostriatal degeneration, postpone the onset of illness or substantially slow disability. Tyrosine hydroxylase plays important role in the synthesis of dopamine. Application of gene therapy increased the production of the corrective enzyme, raised the level of dopamine near the cells that have been deprived of dopamine and partially eliminated the movement disorders in experimental animals.



In another study, glial cell line-derived neurotrophic factor gene therapy was found to slow the dopaminergic neuron cell loss in rat. Transplantation of neurons from substantia nigra of foetal rats containing anti-apoptotic gene corrected some of the Parkinsonian defects in another study. Thus, gene therapy may offer better prospects for the treatment of Parkinson's disease in human in the near future.

Motor neuron diseases

Motor neuron diseases (MND) such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) cause progressive paralysis, often leading to premature death. Neurotrophic factors have been useful as therapeutic agents for MNDs but their clinical use as injected recombination protein was limited by toxicity and poor bioavailability. Adenovirus-mediated neurotrophin-3 gene therapy was found to produce substantial therapeutic effects in the mouse with MND, offering new prospects for the treatment of MND.

Alzheimer's disease

Alzheimer's disease (AD), the most common cause of dementia in adults, affecting nearly 10% of population over 65 years of

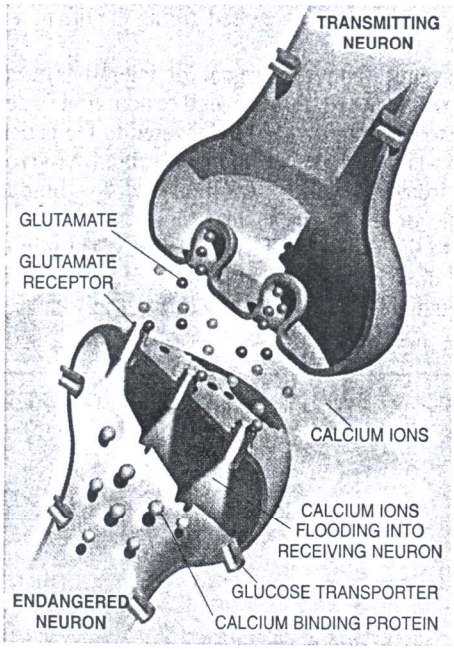


Figure 2. During stroke, too much of the neurotransmitter glutamate *(blue)* accumulates in the synaptic gap. Glutamate receptors *(tan)* then allow calcium ions *(purple)*, which is present in high concentration only extracellularly, to flood into the cell's interior and cause permanent damage to the cell.

age is associated with selective damage of brain regions and neural circuits critical for cognition and memory. Symptomatic treatment with cholinergic drugs temporarily improves cognitive and functional performance without any effect on progressive neurodegeneration. Animal experiments demonstrate that nerve growth factor (NGF) gene therapy may be applied to prevent neurodegeneration by grafts of growth factor producing cells or by direct introduction of growth factor gene.

Stroke

The success of current research with animals indeed sparks the hope that gene therapy offers the prospect of stemming tissue damage during acute neurological crisis such as stroke. Under this condition, the most vulnerable cells - neurons - respond to an extremely powerful neurotransmitter called glutamate. Glutamate induces recipient neurons to take up calcium which causes long-lasting changes in the excitability of synapses (Figure 2). During stroke, neurons are unable to mop up glutamate from synapse or clear calcium that floods into many brain cells resulting in serious damage. This damage then kills cells directly or signals the initiation of internal suicide programmes of neurons (apoptosis). Thus, removal of excess glutamate from the synapses or excess calcium from the neurons form an approach to the treatment of stroke. Animal experiments showed that gene therapy could interrupt this calamitous sequence of events. Glucose-transporter gene therapy in rat lessens the damage of neurons from stroke by supplying sufficient intracellular glucose to pump out calcium from neurons. In another animal study, anti-apoptosis (bcl-2, NAIP) gene therapy was found effective to protect the damage of neurons by inhibiting the apoptosis induced during neurological crisis from stroke.

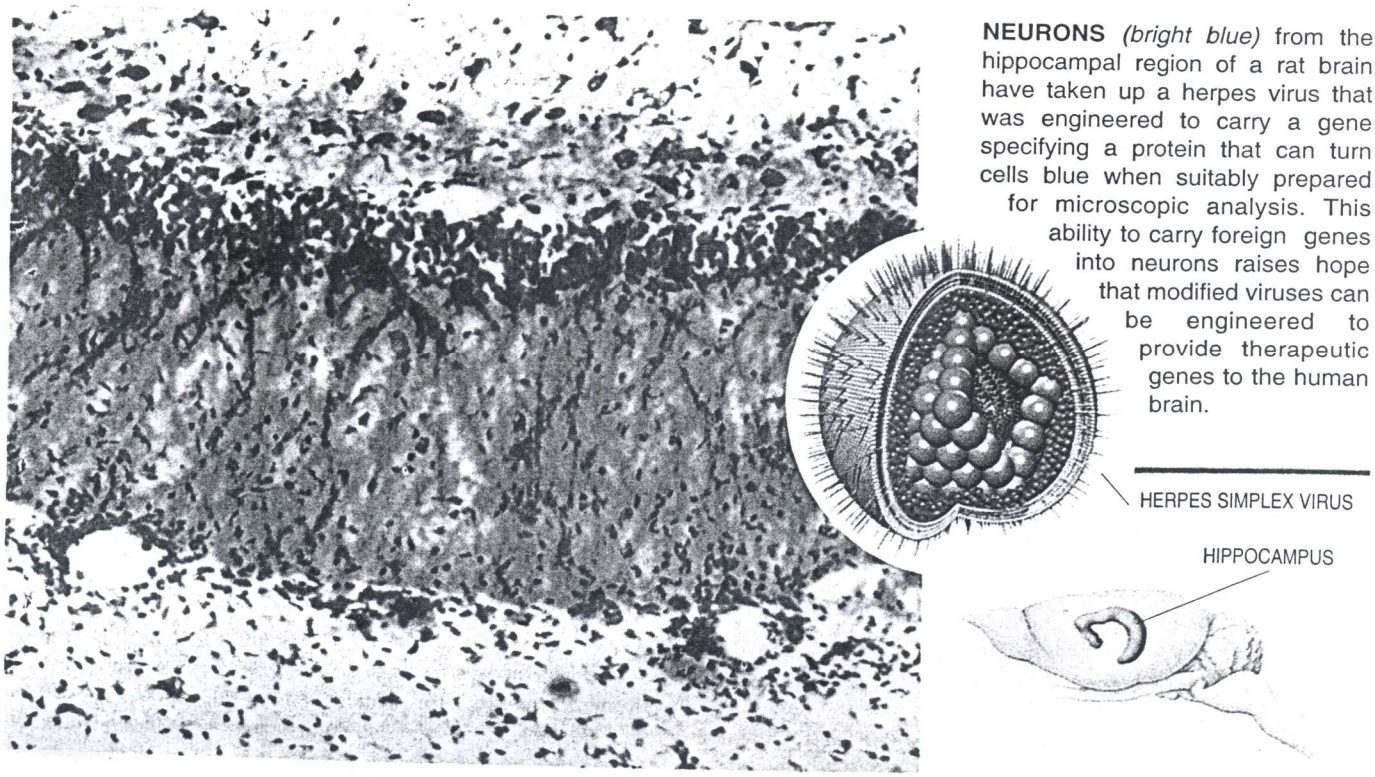
Improving memory and rejuvenating aging brain

Anders Bjorklund and his colleagues at the University of Lund in Sweden applied gene therapy to improve memory in aged rat. These researchers engineered foetal neurons to produce large quantities of nerve growth factor. They implanted these neurons to the brain area that is critical for learning and memory - which slowly degenerates during normal aging. Remarkably, the procedure reversed cognitive decline in aged rats. This success suggests that gene therapy might serve not just to prevent memory loss but to improve memory, sensation and coordination in older people thus helping to rejuvenate aging brain. Such treatments might also be able to make younger people's minds work "better than well".

Conclusion

Despite the many encouraging first steps in applying gene therapy to neurological disorders, hurdles remain. A significant problem persists in engineering viral and non-viral delivery agents and gene delivery systems. Blood brain barrier, anatomic location of brain, vector associated infection and immune response also play important role in the application of gene therapy.

Although there are many technical difficulties to overcome, inserting genes into brain cells may one day offer physicians a better approach to the treatment of neurodegenerative and other neurological diseases.



NEURONS *(bright blue)* from the hippocampal region of a rat brain have taken up a herpes virus that was engineered to carry a gene specifying a protein that can turn cells blue when suitably prepared for microscopic analysis. This ability to carry foreign genes into neurons raises hope that modified viruses can be engineered to provide therapeutic genes to the human brain.

GENE THERAPY

a new horizon for medical science

Dr James Jacob Ribero

Gene therapy may be defined as the introduction of a functioning gene into a cell to reverse a genetic deficiency or to introduce new function to the cell. This definition is especially applicable to monogenic diseases. To include polygenic diseases, the scope of gene therapy is widened to include use of genetic techniques to make cells susceptible to other drug treatment or to enhance immunological mechanisms against infections or malignant diseases.

Gene therapy can be divided into two categories according to the types of cells being genetically altered. Somatic cell gene therapy is applied to genetically alter any cells other than germ cells. Thus it would change individuals' genetic constituents only during their lifetime and would not affect their offsprings. Germ-line gene therapy, on the other hand, is applied to fertilized eggs. This would affect germ cells as well as somatic cells and would be transmitted to future generations. Current gene therapy research is restricted to somatic cell gene therapy due to various technical and ethical reasons.

HISTORY OF GENE THERAPY

1960-1970s	Isolation of genetically marked mammalian cells Uptake and expression of exogenous DNA in mammalian cells
1960s	Role of papovaviruses in cell transformation
1966-70	Public discussion of ethical, scientific and clinical considerations
Early 1970s	Mechanisms of infection and reverse transcription by RNA Tumour viruses
1972	Public discussion of use of transforming viruses for therapeutic gene transfer
1980	Cline human study
1980	Renewed public discussion on the ethics of human gene therapy
1981	Stable in vitro correction of HGPRT deficiency by calcium phosphate-mediated transfection
1981-1982	Development of retroviral vectors
1983	Banbury gene therapy meeting
1983	Complementation of genetic defect and correction of disease phenotype in vitro
1989-1990	First approved human clinical marking studies
Early 1990s	Phase I and II therapeutic gene therapy clinical trials

Dr James Jacob Ribero, MBBS, Executive, MSD

GENE DELIVERY SYSTEMS

To introduce genetic materials into cells a *vector* is employed which acts as a package and ÒinfectsÓ the targeted cells (transfection) and delivers its payload. Gene therapy vectors can be of diverse types, which can be conveniently divided into viral and nonviral types *(Table 1)*.

Ideally a vector should deliver the gene of interest into targeted cells efficiently and specifically with minimum local and systemic side-effects. Currently available vectors have limitations in terms of efficacy, specificity and safety. One important property of vectors is the amount of DNA material they can transfer. This is usually measured in kilobase (kb) and is one of the limiting factors for vector usefulness.

Once inside the cells, the transferred genetic material is expressed and the genetically coded protein is manufactured by the cells. The time period over which this expression of desired protein is carried on is dependant upon whether the delivered genetic material is integrated into the normal genome of the cells or not. Due to lysosomal degradation of non-integrated genetic materials, the time period of protein expression is usually limited to days, weeks or months. In cases of retrovirus and artificial chromosome, it can be virtually permanent and extends into next generation of meiotic daughter cells.

Genes can be delivered via vectors into a patient in two basic clinical methods: *ex vivo* and *in vivo*. In ex vivo method, cells from selected tissue in a patient is removed, exposed to vectors, i.e. transfected, in the laboratory and then the genetically

Table 1. Gene delivery vectors

Viral vectors
Retrovirus
Adenovirus
Adenoassociated virus (AAV)
Others: Herpes simplex virus, Vaccinia virus, Polio virus, Sendai virus, Semliki Forest virus etc.

Non-Viral vectors
Liposome/Lipoplex
Ligand-DNA conjugates
Naked DNA
Particle bombardment
$Ca_3(PO_4)_2$ mediated transfection
Artificial chromosomes: YAC, MAC

modified cells are injected into the patient's body. This method is comparatively common. On the other hand, the in vivo method employs direct injection of a vector into a patient's body, usually into the tissue to be treated or into blood stream.

Viral Gene Delivery System

Numerous viruses have been investigated as potential gene delivery vectors. Among them retrovirus, adenovirus and adenoassociated virus are most common.

Retrovirus: Retroviruses infect dividing cells only and integrate their genome into chromosomes of infected cells permanently. Such integrated genes are replicated and passed to all future generations of the transfected cells. Limitations of retrovirus vectors include their low DNA carrying capacity (4-5 kb) and the possibility of mutagenesis.

Adenovirus: They can infect a wide variety of cells including non-dividing (quiescent) ones. The gene delivered by adenovirus is not integrated into infected cell's chromosome, so the expression of gene products is transient. They have a large capacity for gene delivery. They are relatively safe but carry the risk of invoking an inflammatory response from the host body.

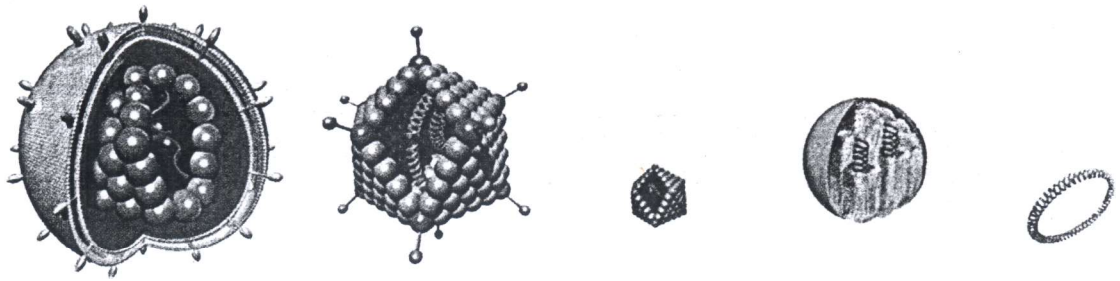
Adenoassociated virus (AAV): AAV is a defective virus (parvovirus) that requires help from adeno- or herpes virus for replication. AAV has the ability to integrate at a specific site on human chromosome 19, which lessens the risk of mutagenesis.

Non-viral Gene Delivery System

Non-viral gene delivery system embraces a medley of different vectors based on dissimilar principles. Some of them are useful for experimental purposes only.

Liposomes/Lipoplexes: These are complexes of cationic liposomes and negatively charged plasmid DNA. They are taken up by targeted cells by endocytosis. Liposomes have wide safety margin and large DNA carrying capacity. The length of time for gene expression is variable and short due to lysosomal

Some Viral Vectors Under Study



	Retroviruses	Adenoviruses	Adenoassociated Viruses	Liposomes	"Naked" DNA
Some Potntial Advantages	Integrate genes into host chromosomes, offering chance for long-term stability	Most do not cause serious disease; large capacity for foreign genes	Integrate genes into host chromosomes; cause no known human diseases	Have no viral genes, so do not cause disease	Same as for liposomes; expected to be useful for vaccination
Some Drawbacks of Existing Vectors	Genes integrate randomly, some might disrupt host genes; many infect only dividing cells	Genes may function transiently, owing to lack of integration or attack by the immune system	Small capacity for foreign genes	Less efficient than viruses at transferring genes to cells	Inefficient at gene transfer; unstable transfer; unstable in most tissues of the body

degradation of liposomes in the transfected cells.

Artificial Chromosome: This is an artificially prepared complete chromosome consisting of desired genes with accessory regulatory genes and components for stable self-replication (telomere and centromere). Artificial chromosome would overcome limitations of the amount of DNA material that can be delivered by other vectors. They have stable and tissue specific expression of gene products as have been demonstrated by using Yeast Artificial Chromosome (YAC) in transgenic mice. Major effort is underway to produce Mammalian Artificial Chromosome (MAC) and already functional truncated X chromosome and reduced Y chromosome have been successfully produced.

CLINICAL APPLICATIONS OF GENE THERAPY

At first gene therapy was envisaged as a replacement therapy for monogenic disorders. The information gained from gene transfer and interventional genetics is now being applied to polygenic and multifactorial disorders.

Cystic Fibrosis

This is a monogenic recessive disorder with a carrier frequency of approximately 1 in 25 in the UK. Here the defective CFTR (cystic fibrosis transmembrane conduction regulator) gene leads to abnormality in chloride channels especially in lungs, pancreas and biliary system. Already phase I clinical trials have been undertaken to treat this disease. An American clinical trial using adenovirus vector and a British trial using liposome to deliver functional CFTR gene have shown promising results.

Ischaemic Heart Disease

Patients with familial hypercholesterolaemia develop the disease due to a defective low-density lipoprotein receptor (LDLR) gene. They develop atherosclerotic changes during childhood and may die of ischaemic heart disease in their adolescence. A trial of gene therapy for this disorder using ex vivo method has been recently undertaken and showed significant lowered cholesterol level.

Cancer Gene Therapy

Several approaches to cancer gene therapy are being explored. First, immune responses to tumour are being enhanced. Secondly, genes are being inserted into tumour cells to evoke cell suicide (apoptosis). Lastly, methods are being developed to modify tumour suppressor genes or anti-oncogenes.

Immunological approach of cancer gene therapy uses transfer of genes encoding cytokines, antigens or major histocompatibility complex (MHC)-molecules into tumour cells by ex vivo or in vivo method to heighten the immunological response against the tumour cells. Malignancies such as melanoma, renal cell carcinoma and lymphomas are particularly amenable to this strategy since they express tumour-specific antigens.

'Cell suicide' involves the insertion of a herpes simplex virus thymidine kinase (HSV-TK) gene. Mammalian cells contain TK gene that can only phosphorylate a thymidine nucleotide but cannot add a phosphate to the nucleoside base T (thymine). On the other hand, HSV-TK can phosphorylate a nucleoside base. Thus any cell infected with HSV can be killed by exposing it to nucleoside agents such as ganciclovir which, when phosphorylated, interfere with DNA synthesis. To treat brain tumours retroviral vectors containing HSV-TK gene are injected into the vicinity of the tumour. Since only the malignant cells are dividing, the vector should be targeted to the tumour. Ganciclovir is then administered, which causes selective destruction of the transfected tumour cells. In animal experiments complete eradication of tumours has been observed though only some 20% of the tumour cells carried the HSV-TK gene.

Tumour suppressor genes are being inserted into human tumours. One protocol involves inserting a normal *p53* gene into non-small cell lung carcinomas that are *p53* defective. In, another antisense DNA is injected to try to suppress the activity of activated oncogenes, in this case k-*ras* in lung carcinoma.

Other application of gene therapy

Several systems are being explored for the delivery of hormones, coagulation factors, anticoagulants and other therapeutic agents. When their genes are introduced into fibroblasts or vascular endothelial cell, their products can be detected in the blood for varying periods of time. Recent studies in mice suggest that myoblasts may be particularly advantageous target cells for the delivery of recombinant proteins. They fuse randomly with other muscle fibres in their vicinity and become an integral part of vascularised muscle tissue; human growth hormone has been detected in the circulation of mice treated this way for up to 6 months.

Gene Therapy: A New Horizon

Gene therapy is one of the most exciting and fast moving fields of medical research. The concept that genetic disorders and even some infectious, degenerative or malignant diseases may be amenable to correction at a genetic level is an entirely new approach to therapeutic intervention. From being a fanciful idea, gene therapy has progressed over the last 10 years to become accepted as a concept within the scientific and medical communities. Gene therapy truly opens up a new horizon for medical science. ■

MANAGEMENT

# of thalassaemia and promising results in gene therapy

Dr Syed Khairul Amin

Thalassaemia is an inherited disorder of the blood that is passed on from parents to their children.

There are two principal forms of Thalassaemia - Thalassaemia minor and thalassaemia major.

Thalassaemia minor is the heterozygous form of the disease. In this condition the patients are free of any disease symptoms. Their life span is normal.

Thalassaemia major is the homozygous and serious form of the disease. This condition is associated with severe anaemia and considerable disability. Thalassaemia intermedia is also homozygous state of the disease but the symptoms are modified and becomes milder by the presence of DNA polymorphism (G-gamma x mnl).[1]

Haemoglobin E/Beta-thalassaemia is the commonest thalassaemia in Bangladesh. The clinical approach to haemoglobin E/Beta-thalassaemia resembles that for homozygous Beta-thalassaemia. The most important clinical difference between homozygous Beta-thalassaemia and Hb E-beta-thalassaemia relates to the oxygen affinity of the predominant haemoglobin. In thalassaemia major this is Hb F which has high oxygen affinity and release oxygen poorly to the tissues. By contrast in Hb E/Beta-thalassaemia the predominant haemoglobin is Hb E which has low oxygen affinity. Patients can therefore tolerate a low thalassaemia level better than homozygous beta thalassaemia and Hb around 7g/dl often permits acceptable growth and quality of life.

Treatment for thalassaemia mainly focus on two aspects: Blood transfusion every 3-4 weeks interval as long as the child survives and regular chelation therapy to remove excess iron as long as the child lives. The best chelation is done by Desferal. The recommended method is slow subcutaneous infusion over 8 to 12 hours at least five days in a week. This is a very expensive treatment. Deferiprone is an iron chelator that can be taken by mouth. It is less safe than Desferal and its long-term safety and efficacy are unknown.[2]

In recent years preparations like Butyrate[3] and Erythropoietin[4] have been tested for their ability to raise haemoglobin level in thalassaemia but with disappointing results.

Dr Syed Khairul Amin, DCH (Glasgow), MRCP (UK) Associate Professor, Bangladesh Institute of Child Health, Dhaka.

Splenectomy becomes necessary for the majority of low-transfused patient within first 10 years of life because hypersplenism is common and progressive, and often leads to death.[5]

The only curative treatment for thalassaemia is a bone-marrow transplantation. This procedure is very expensive and not without risk and failure. Even more is the difficulty in getting a fully compatible donor.

Researchers at the Columbia University College of Physicians and Surgeons in New York have succeeded for the first time in demonstrating the long-term transfer and high-level long-term expression of normal human beta globin gene in an animal model.

Dr. Arthur Bank and his colleagues at Columbia put a human beta gene into a safe retrovirus, added the virus to mice bone-marrow cells in vivo and transplanted the modified cells into mice. The presence to the human beta globin gene could be detected up to eight months later with high levels of expression. That level of expression could be enough to ameliorate, if not cure, the anaemia of patients with beta thalassaemia.

Researchers are continuing to investigate better gene transfer systems in mouse models of beta thalassaemia and developing better ways to transfer retroviruses into human haemopoietic stem cells.

Their results may lead to clinically successful gene therapy for beta thalassaemia. ■

References:

1. Racep S, Petrou M, Old J, Wonke B, Porter J, Modell B. Relationship between the severity of beta Thalassaemia syndromes and the number of alleviating mutations. *European Journal of Heamatology* (in press) 1996.
2. Al Rafaie FN, Hershko, Hoffbrand AV *et al.* 1995. Results of long-term deferiprone (LI) therapy: a report by the International Study Group on Oral Iron Chelators. *British Journal of Haematology* 91: 224-229.
3. Sher GD, Ginder GD, Little J *et al.* 1995. Extended therapy with intravenous arginine butyrate in patients with betahaemoglobinopathies. *New England Journal of Medicine* 332: 1606-10.
4. Loukopoulos D, Voskaridou E *et al.* 1995. Response of Thalassaemia intermedia to hydroxyurea alone or in combination with high doses of recombinant human erythropoietin. (Submitted for publication)
5. Modell B, Berdoukas V. 1984. The clinical approach to Thalassaemia. Grune and Stratton, New York and London.

THE FRACTURE of the neck of the femur

A REVIEW OF THE RELEVANT ASPECTS AS A GUIDE IN CLINICAL PRACTICE

Dr MM Anwar

PROLOGUE

No single article or a textbook has been able to cover all-important aspects of the femoral neck fractures. It has been realized that such matter as said needs to reach the residents as well as those who are in clinical practice covering most of these aspects, if not all. This is easier said than done. A thumbnail sketch has been prepared from different course lectures, textbooks and journals with a view to meeting this demand as precisely as possible. This article is based on known facts to many, yet it is intended to help one remind the same as a guide which has received commendation in the yearly meeting at the University of Birmingham, England in 1996.

INTRODUCTION

Fractures of the neck of the femur (FNOF) were recognized over 400 years ago by Ambrose Pare, the famous French Surgeon, but Sir Astley Cooper (1768 - 1841) of Guy's Hospital, London was the first to delineate clearly between fractures of the femoral neck, or intracapsular fractures, and other fractures and dislocations about the hip.

There has been a defeatist attitude in the management of these fractures, which has long been reflected by the quotation, "we come into the world under the brim of the pelvis and go out through the neck of the femur".

The fractures of the neck of the femur have been unique for its anatomic characteristics that render the fractured hip vulnerable to a number of complications.

CONSIDERATION OF ANATOMY RELEVANT TO HIP FRACTURES:

In Adults

THE HIP JOINT

The capsule is attached anteriorly at the intertrochanteric line, however, posteriorly the lateral half of the femoral neck is extracapsular. The followings are some essential features of note:

1) The intracapsular portion has essentially no cambium layer in its fibrous covering to participate in peripheral callus formation, therefore, healing in the femoral neck area is dependant on endosteal union alone.
2) Unless the fracture fragments are carefully impacted, synovial fluid can lyse blood clot formation and thereby destroy another mode of secondary healing by the prevention of the formation of cells and scaffolding that would allow for vascular invasion of the femoral head.
3) The pattern of the vascular supply also has some bearing on the complications occurring after having sustained the fracture.

The vascular supply to the proximal end of femur:

The proximal femur is supplied by three groups of arteries:
a) An Extracapsular Arterial Ring located at the base of the femoral neck formed by
- The ascending branch of the lateral circumflex femoral artery anteriorly,
- A large branch of medial circumflex femoral artery posteriorly and
- Inferior division of the superior gluteal artery and inferior gluteal artery of the Internal Iliac Artery.

b) Ascending Cervical Branch of the Extracapsular Arterial Ring and
c) Arteries through the Ligamentum Teres (supply a small area of subsynovial circulation but often inadequate to assume the major nourishment of the femoral head following a displaced fracture if all other sources of supply are interrupted); also known as Medial Epiphyseal Artery.

The Ascending Cervical Branches from the Extracapsular Ring pass upward under the synovial reflections and fibrous prolongation of the capsule toward the articular cartilage that demarcates head from neck. These are known as Retinacular Arteries. This close proximity of the Retinacular Arteries to bone puts them at risk of injury in any fracture of the neck.

The Retinacular arteries are - Anterior, Posterior, Medial and Lateral. The Lateral Retinacular provides most of the blood supply to the femoral head & neck. The blood supply to the head thus comes from chiefly the Retinacular vessels, arteries through the Ligamentum Teres and the interosseous cervical vessels.

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When a femoral neck fracture occurs the interosseous cervical vessels are disrupted and the supply depends on the rest of two sources. The Retinacular vessels are very important but are also at risk during an injury that accounts for a high incidence of avascular necrosis in case of neck fractures, esp. when displaced.

In Children

They are extremely rare, as compared to hip fractures. In adults with osteoporotic bone, it has been estimated that incidence of children's hip fractures is less than 1% of adult hip fractures. Trueta's observations refined and augmented by Chung and Ogden about the blood supply in case of children are as follows:

1. At birth, the femoral head is supplied by the metaphyseal vessels that come from the medial and lateral circumflex vessels.
2. Vessels through the ligamentum teres; the supply is virtually negligible until the age of 8 years, after this age the supply is only 20% as an adult.
3. Between birth and the age of 4:

a) Diminution of metaphyseal vascular supply by the medial and lateral circumflex arteries as the cartilaginous growth plate develops, which is virtually non-existent at age 4.

b) The predominant supply starts being in existence by the postero-superior and postero-inferior branches of the medial circumflex artery, which constitute later, the retinacular arterial system as described in the adult part.

4. At the age of 3 or 4 the lateral of the postero-superior vessels appear to predominate and to supply all the anterior and lateral portions of the head of the femoral epiphysis, although both the postero-superior and postero-inferior vessels persist throughout life to supply the head.
5. Between 4 and 8 years of age, the head is supplied by :

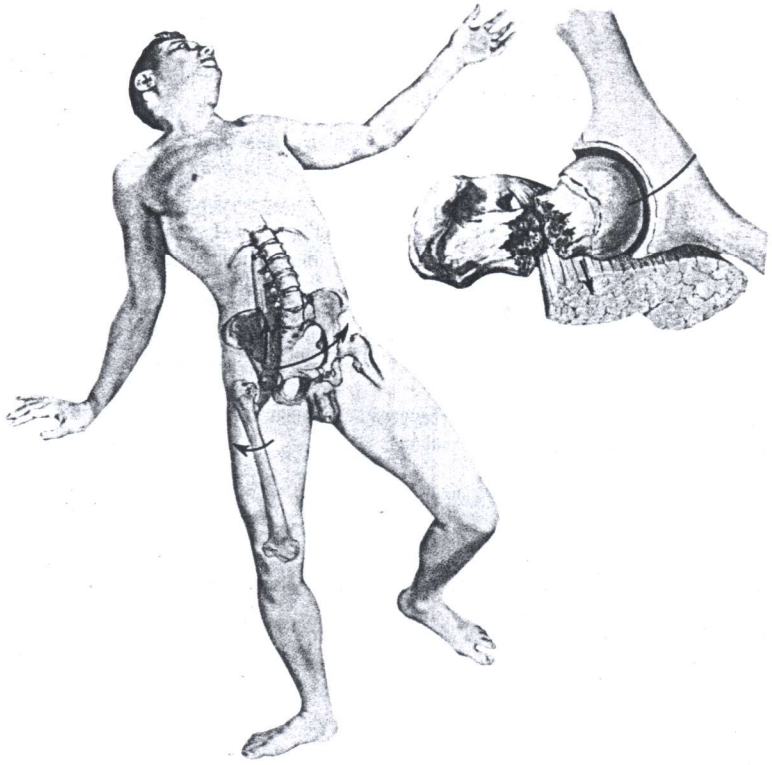
a) Retinacular vessels.
b) Vessels through the Ligamentum Teres.

So, the retinacular supply is the only dependable supply to the head after the age of 3 or 4 since the supply through Ligamentum Teres is negligible and cessation of supply by the metaphyseal vessels. For this reason, this age group is vulnerable to avascular necrosis if there is any vascular insult.

MECHANISM CAUSING FEMORAL NECK FRACTURE

The fracture may be brought about by sudden violence or oft-repeated stress. Although the latter has not yet been fully accepted, cyclical loading of the femoral neck has been seen to cause it (Urovitz, Clin Orth 1977; Todd JBJS 1972).

The violence causing the fracture may be a direct one as in direct blow to the greater trochanter or an indirect one sustained by an external rotation mechanism (Kocher 1896) as depicted below:



INDIRECT MECHANISM CAUSING FEMORAL NECK FRACTURE:

TRAUMA: Major and minor injuries are both responsible for fracture causation.

AGE: Most commonly in elderly patients with the average age of 76 years. It is rare in children.

SEX: Females are three times more frequently affected then males.

RACE: Black people, especially the American Negroes and South Africans suffer less.

BONE QUALITY: Poor bone quality pre-disposes to failure with even minor trauma.

PHYSICAL ACTIVITY: Stress fractures in joggers or military recruits.

IRRADIATION

DISEASES: Osteoporosis, Osteomalacia, Osteoarthritis (fracture protective disease for the neck i.e. OA $\propto$ 1/FNOF), Paget's disease, Parkinson's disease, Paralytic conditions i.e. hemiplegia, Renal osteodystrophy, Unicameral bone cyst, Fibrous dysplasia, Neglected septic arthritis and Metastatic disease.

CONVULSIONS: These may predispose to bilateral affection as does the vigorous physical activity and also the effect of hypoparathyroidism.

CLASSIFICATION

A number of classifications are available which again denotes existence of debates in the field. The followings are some examples:

AETIOLOGIC CLASSIFICATION
- Single violence-produced fracture
- Violence of insufficient magnitude to produce fracture of a normal bone
  - # by oft-repeated violence
  - Stress/Fatigue # of a normal bone
  - Fracture of bone already weakened by disease (Pathological #)

ON THE BASIS OF PT.'S AGE
- In children (1% of adults)
- In adults

ON THE BASIS OF FRACTURE CHARACTERSTICS

On the basis of anatomic location

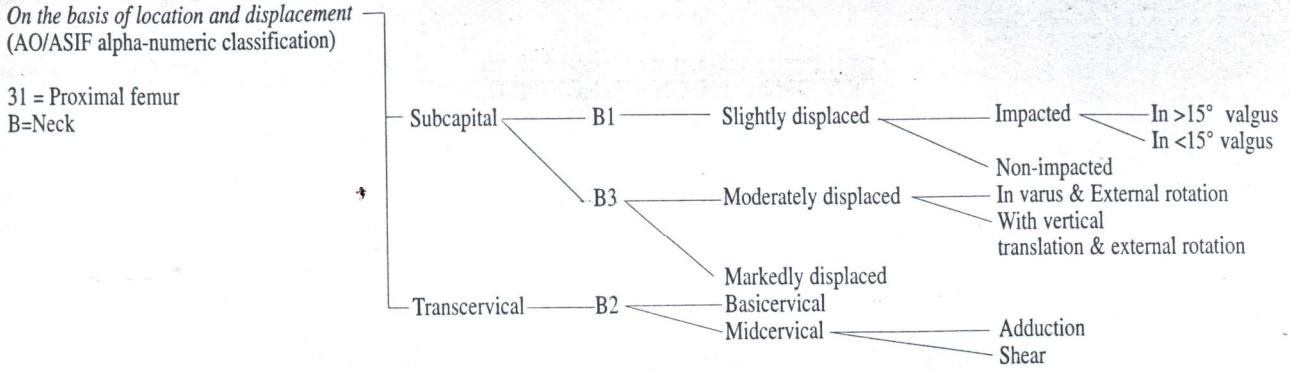
- Cooper's classification
  - Intracapsular
    - Subcapital
    - Transcervical
  - Extracapsular/Basilar
- Bank's classification
  - Intracapsular
    - Subcapital
      - Classical subcapital
      - Wedge subcapital
    - Inferior beak
    - Mid-neck
  - Extracapsular / Basilar
- Children's fracture (Delbet's classification)
  - Transepiphyseal (6-10%)
    - Without dislocation
      - Undisplaced
      - Displaced
    - With dislocation (More prevalent at 2-3 yrs or in adolescents of about 14 yrs)
  - Transcervical (45-50%)
  - Cervico-trochanteric (30-40%)
  - Intertrochanteric (10-20%)

On the basis of the direction of fracture (Pauwell's classification)
- Type I 30 degree angle
- Type II 50 degree angle
- Type III 70 degree angle

Demerits : 1. Direction of # line could be altered by changes in the limb or beam position.
2. Most (85%) of fracture surfaces are between 45° and 60°.

On the basis of displacement of fracture fragments

- Garden's classification
  - Garden I : a) Incomplete or impaced
    b) The trabeculae of the inferior neck are still intact (Abducted impaction fracture).
  - Garden II : a) Complete fracture.
    b) No displacement.
    c) Trabeculae are interrupted by a fracture line across the entire neck
  - Garden III : a) Complete fracture.
    b) Partial displacement (< 50% according to Kyle)
    c) Trabeculae do not line up with those of the acetabulum
  - Garden IV : a) Complete fracture
    b) Complete displacement (>50% displacement according to Kyle)
    c) Trabeculae of the head line up with the acetabulum.
- Tronzo's classification
  - Stable (Fragments are closely connected)
    - Undisplaced
    - Slightly displaced
      - In varus
      - In valgus
    - Impacted
  - Unstable (Fragments are disconnected)
    - Valgus with more than a few degrees of lateral displacement
    - Varus with varying degrees of displacement in both planes



DIAGNOSIS

1. History
2. Pain. This may be slight in case of stress and impacted fractures.
3. Inability to bear weight. The pt. may still walk with a limp if the fracture is undisplaced.
4. Shortening of the limb. Less obvious in undisplaced fractures.
5. External rotation. Less obvious in undisplaced fractures or displaced ones than extracapsular fractures.
6. Radiographic evidence of fracture.

- 50% young patients with FNOF have sustained multiple trauma and may have other serious injuries.
- 20% of the young patients with FNOF have an ipsilateral femoral shaft fracture.
- FNOF may be missed in 40% patients who have been attended for femoral shaft fracture. (Kyle RF JBJS 76-A 1994 June)

SURVIVAL/MORTALITY

1. In the past mortality from FNOF was as high as 40%. Recently, this ranges between 3% and 40%.
2. Survival is best measured at the one-year level. If the patient survives the first year, his longevity becomes equal to that of the normal population of the same age group.
3. Factors affecting survival or mortality are: -a) Age, b) Mental status and c) Postoperative confusion.

DEFINITION OF AN IMPACTED FRACTURE

DEFINITION BY TRONZO

It is an incomplete fracture with intact cortex usually inferior one rendering an inherently stable fracture complex by a firm jamming of the proximal fragment into the distal one causing an abduction displacement without any radiographic evidence of such displacement on the lateral view.

CRITERIA TO DECLARE A FRACTURE AS IMPACTED FOR THE PURPOSE OF NONOPERATIVE (CRAWFORD) TREATMENT

1. X-Ray evidence of abduction on AP and no displacement on lateral view.
2. No shortening of the limb
3. No external rotation of the limb
4. No discomfort on active and passive range of motion (ROM) of the hip
5. Ability to perform active internal rotation of the limb
6. Cooperative patients who are agile and alert

OVERALL MORTALITY RATE: 16%-26%
LOSS OF REDUCTION DESPITE SELECTION OF PTs.: From 8% to 20%

MANAGEMENT OF FRACTURE NECK OF FEMUR

It includes:
1. General Management
2. Definitive Management
3. Rehabilitation

DEFINITIVE MANAGMENT

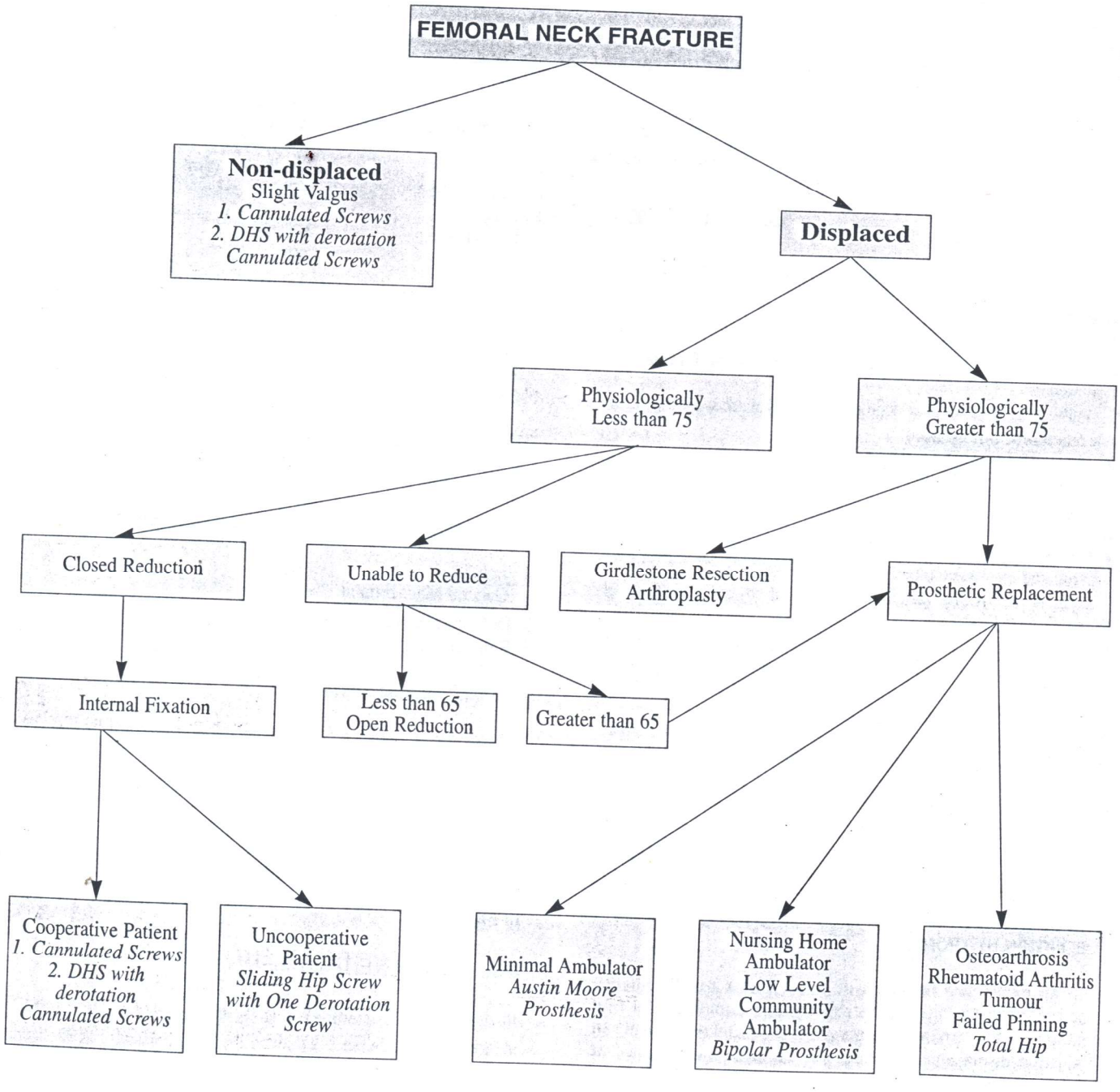
The options for treatment in fracture neck of femur are as follows:

In Children:
1. Hip spica
2. Closed reduction: Hip spica, Internal fixation
3. Open reduction and internal fixation.

In Adults:

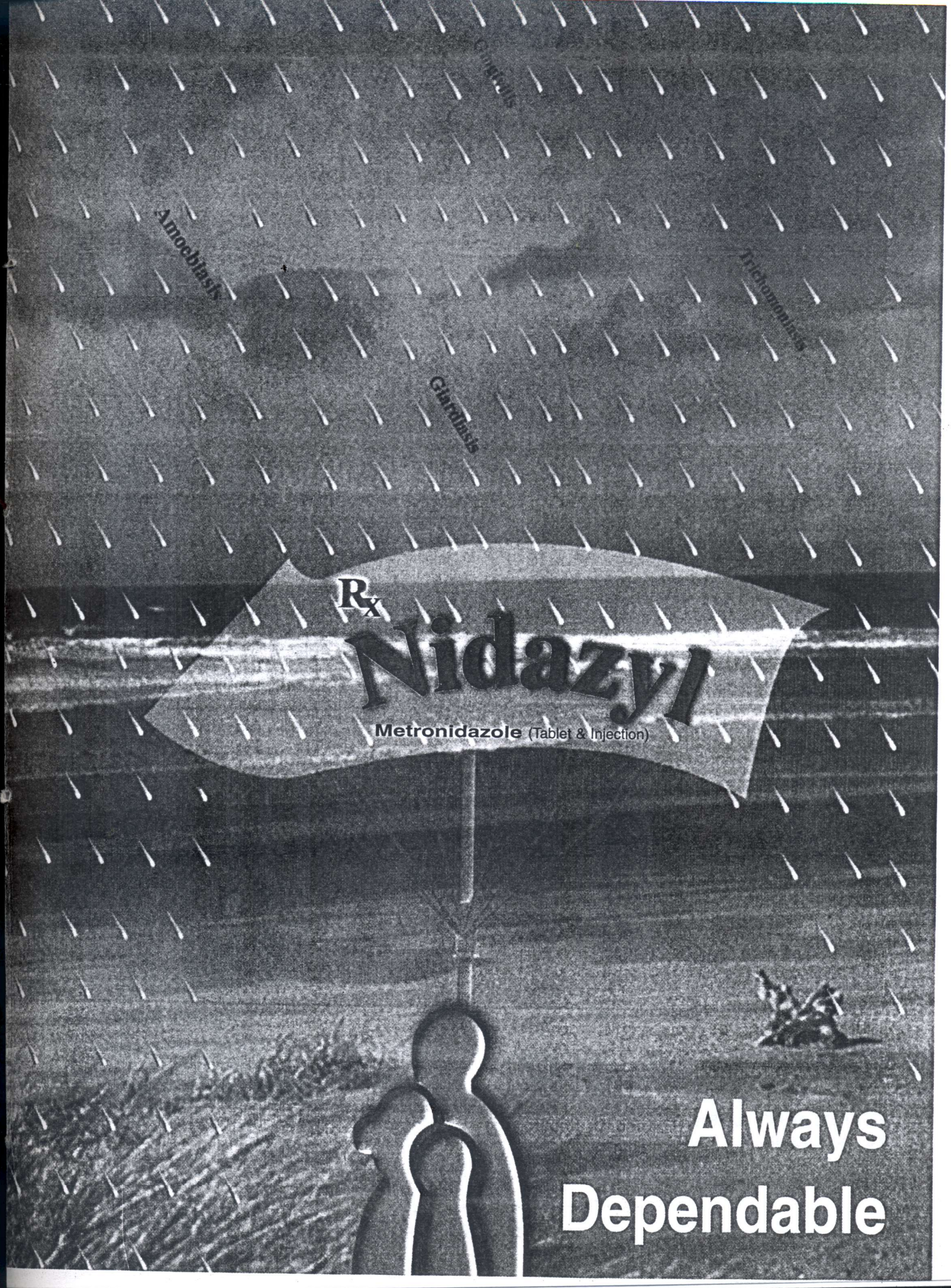
NON-OPERATIVE TREATMENT

In the developed countries, this form of management is rarely adopted unless dictated by absolute contraindications such as medical conditions and very stable impacted fractures. If, adopted, rest in bed is immediately followed by nonweight bearing (NWB) as soon as comfortable for 8-12 weeks (until radiographic evidence of healing). Nonetheless, fixation is again chosen if secondary displacement (delayed operation after secondary displacement does not affect the rate of mortality, nonunion or avascular necrosis) occurs.



A modified flow chart for the definite management of the fracture neck of femur (from: Kyle R.F.: 1993)

(Continued to page no. 21)


Amoebiasis
Giardiasis
Trichomoniasis
℞
Nidazyl
Metronidazole (Tablet & Injection)
Always
Dependable

PROLIFERATION OF intrahepatic bile-duct epithelium in biliary atresia :

A USEFUL PREDICTOR OF CLINICAL OUTCOME

Dr Moazzem Hossain, Dr Osamu Murahashi, Dr Hisami Ando, Dr Kentiro Kaneko, Dr Takahiro Ito

ABSTRACT

Proliferating cell nuclear antigen (PCNA) and transforming growth factor α (TGFα) are considered as markers of cell proliferation. The expression of PCNA and TGFα was evaluated immunohistochemically using anti-PCNA antibody and TGFα in 31 patients with biliary atresia (BA) (15 jaundice-free and 16 with persistent jaundice) and 6 control infants. The labeling indices (LI) for PCNA- and TGFα-positive bile-duct epithelium in BA were 14.1±14.0% and 51.4±33.7%, respectively, which was significantly higher than in the controls (P <0.01). In BA, the number of PCNA-immunoreactive cells was higher in the peripheral bile ductules than in the central bile ducts of the portal tract (P <0.01). LI was not related to patient age at the time of hepatic portoenterostomy in two groups divided at the age of 60 days. Patients in the persistent jaundice group had greater expression of PCNA and TGFα (21.7 ± 16.0% and 76.9 ± 20.7%, respectively) compared to those in the jaundice-free group (6.0±2.7% and 24.3±20.9%, P <0.001). PCNA and TGFα expression in the bile-duct epithelium of the portal tract was closely related to prognosis in BA patients, and thus could be useful as a prognostic marker.

INTRODUCTION

Recent progress in immunology has made many new and useful methods of morphologic studies of various cellular proteins available. Proliferating cell nuclear antigen (PCNA) and transforming growth factor α (TGFα) are two such substances. PCNA/cyclin is an auxiliary protein of DNA-polymerase δ, and its synthesis is directly related to the proliferative state of the cell.[4-5,7] It can be detected in dividing and newly formed cells.[5] TGFα on the other hand, has been demonstrated to be a secreted polypeptide available in both normal and transformed cells.[15,19] It plays an important role in cellular proliferation and malignant transformation via autocrine stimulation of growth.[15,19] These two proliferating agents are widely used as markers in studying various types of neoplasms. [6,20]

Kasai's hepatic portoenterostomy has resulted in long-term survival in approximately 25%-30% biliary atresia (BA) patients.[10-12] Some less fortunate patients have developed portal hypertension and progressive hepatic insufficiency and eventually died.[1,13] Although age at operation, histologic alterations of the hepatic parenchyma, and episodes of postoperative cholangitis were thought to be related to the clinical outcome[12], clinical experience has shown that none serve as reliable indicators of prognosis. Our previous studies indicated that intrahepatic bile ducts are one of the most important factors that determine the outcome of BA patients.[8,9] To investigate the pathological changes of the bile ducts in more detail, we used monoclonal antibodies for PCNA and TGFα in immunohistochemical studies of biopsied liver specimens taken at the time of portoenterostomy. This is the first application of these proteins to study the intrahepatic bile ducts and ductules in BA, and expression of these proteins correlate well with the clinical outcome of BA patients.

MATERIALS AND METHODS

Hepatic tissue was obtained by surgical biopsy from the right anterior hepatic segment of 31 BA patients at hepatic portoenterostomy. Six liver specimens from autopsied infants without hepatobiliary disease were used as histologically normal controls. Tissue sections of 3 μm were prepared from 10% formalin-fixed liver specimens. For immunohistochemical study, they were deparaffinized using xyline and graded ethanol and then incubated in methyl alcohol containing 1.5% hydrogen peroxide for 20 min to block the endogenous peroxidase activity. For PCNA immunostaining only, sections were then incubated in 2N HCI for 30 min at 20°C, followed by washing in two successive baths (5 min each) in 0.1 mol/L borax at pH 8.5, then incubated in 0.5% tween 20 in phosphate buffer solution (PBS) for 10 min and washed in PBS twice, for 10 min each.

The indirect peroxide-labeled antibody method was applied for immunohistochemical staining. The monoclonal antibodies for PCNA (DAKO, PC-10, Denmark) and TGFα (Oncogene Science, Uniondale, NY) were used as first antibody in 1:400 and 1:200 dilutions, respectively. Tissue sections were then allowed to react for 12 h at 40°C. After washing with PBS, anti-

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mouse IgG was conjugated with horseradish peroxidase (MBL, Japan) and used as a second antibody in 1:400 dilution and allowed to react for 3 h. PBS was used as a substitute for primary antibody for the negative controls, which confirmed the specificity of the procedure. Bound peroxidase activity was visualized with 0.25% 3,3-diaminobenzidine solution containing 10 mM hydrogen peroxide. Sections were allowed to react for 3 min, after which they were counterstained with 10% methyl green solution and mounted for microscopic studies.

For quantitative evaluation of the bile-duct epithelium of haematoxylin-eosin and immunostained sections, at least 4-10 portal areas were investigated. Centering the interlobular artery, we divided the bile ducts in the portal tract equally into three groups: central, mid, and peripheral, according to their locations. We excluded portal tracts where the interlobular artery was ill defined. Positive and negative immunoreactive cells were counted in the sections, regardless of the degree of reactivity with monoclonal antibodies for PCNA and TGFα; nuclei that were stained brown were considered positive. Sections were examined without any prior knowledge of the patient's prognosis. The labeling index (LI) was applied as previously described[17], corresponding to the number of nuclei positive for PCNA and TGFα among a total of 1,000 nuclei and expressed as percentage of positive cells.

Ten patients were operated upon before 60 days of age and the remaining 21 after 60 days. Fifteen patients with serum bilirubin levels less than 1 mg/dl were considered to have cleared jaundice and the remaining 16 were never completely anicteric during the postoperative period. Statistical analysis was performed using the unpaired student t-test. Differences were considered significant if P was less than 0.05. Result were expressed as mean ± SD.

RESULTS

Histologic findings

Conventional histologic studies of the liver specimens showed findings characteristic of BA. There was widening of the portal tracts, cholestasis in the bile ductules, infiltration of mixed inflammatory cells, fibrosis, and proliferation of bile ductules with or without intraluminal accumulation of cellular debris or bile plugs. Nonspecific cholangitis with degeneration and necrosis was always observed in the epithelial lining of bile ducts although the degrees varied.

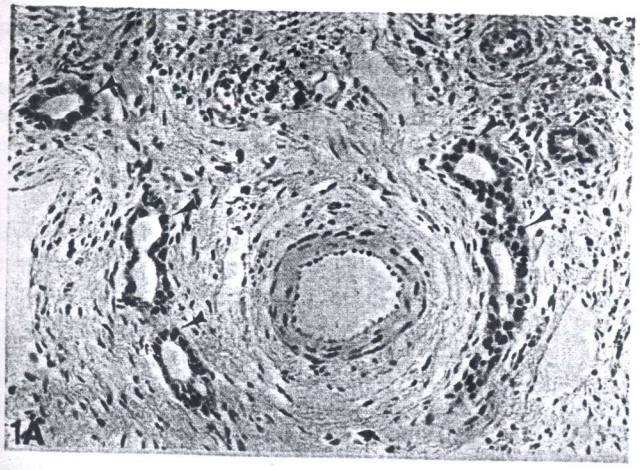
Immunohistochemical findings

PCNA and TGFα were found diffusely in the bile-duct epithelium of all 31 patients with BA. PCNA was detected in the bile duct epithelium of 1.32 ± 0.35% of the normal controls compared to 14.1±14.0% of BA patients (P <0.02), while TGFα was found in 0.32±0.42% and 51.4±33.7% (P <0.001), respectively. Although PCNA immunoreactive cells were observed in central, mid, and peripheral bile ducts in the portal tract (*Table 1*), their numbers were less in the central than the peripheral ducts (P <0.02). No remarkable difference in TGFα expression was observed among the three groups of bile ducts (*Table 1*).

Table 1. Distribution of proliferating cell nuclear antigen (PCNA) and transforming growth factor α (TGFα)-immunoreactive bile duct epithelium in the portal areas of 31 patients with biliary atresia (BA)

Nos. of positive cells	Central duct	Mid-duct	Peripheral duct
PCNA	8.8±9.1[a]	14.3±15.3	16.7±16.7[a]
TGFα	52.1±35.1	52.0±35.1	53.7±35.8

[a] P <0.02



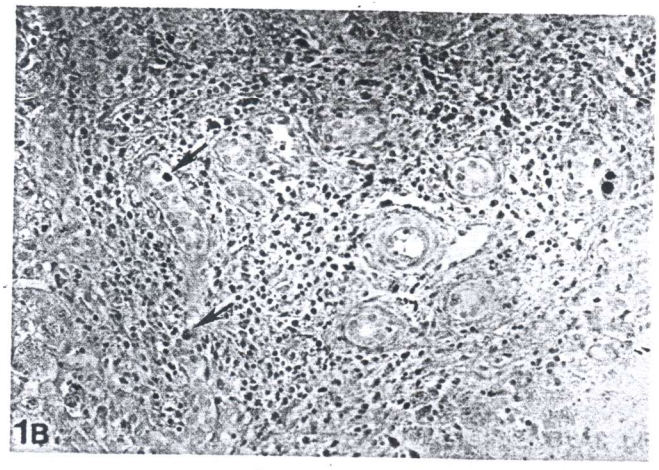


Fig. 1A, B. Microphotographs showing expression of proliferating cell nuclear antigen/cyclin in portal bile-duct epithelium from **A** a jaundice-persistent patient (labeling index [LI] = 78%) (*arrowheads*) and **B** a jaundice-free patient (LI = 4%) (*arrows*).

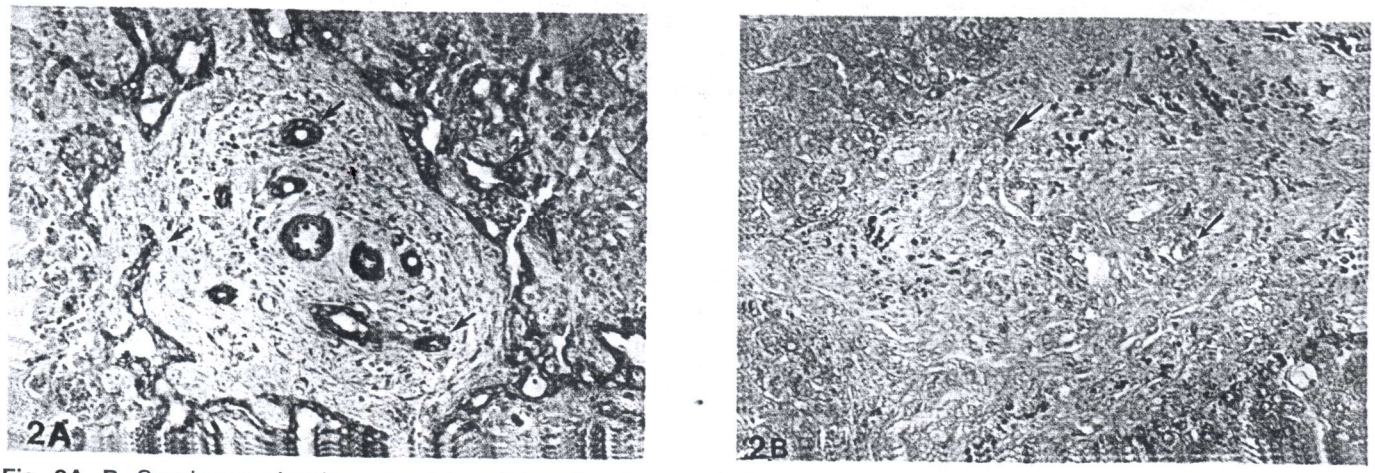


Fig. 2A, B. Specimens showing expression of transforming growth factory α in portal bile-duct epithelium from **A** a jaundice-persistent patient (labeling index [LI] = 97% (*arrows*) and **B** a jaundice-free patient (LI = 3.55) (*arrows*).

Relation to clinical outcome

Patients who never became anicteric had greater expression of PCNA in the portal-tract bile-duct epithelium, (Fig. 1 A) compared to jaundice-free patients (Fig. 1 B). The LIs of PCNA expression in jaundice-persistent and jaundice-free groups were 21.7 ±16.0% and 6.0 ± 2.7%, respectively (P <0.001). Expression of TGFα was also higher in jaundice- persistent than jaundice-free patients, (Fig. 2A and B). LI differed significantly between the two groups of patients, at 76.9 ± 20.7% and 24.3 ± 20.9%, respectively (P <0.001). A highly significant association was observed between the expression of both PCNA and TGFα and the clinical outcome: jaundice-persistent patients showed higher expression of PCNA and TGFα than jaundice-free patients (Fig. 3).

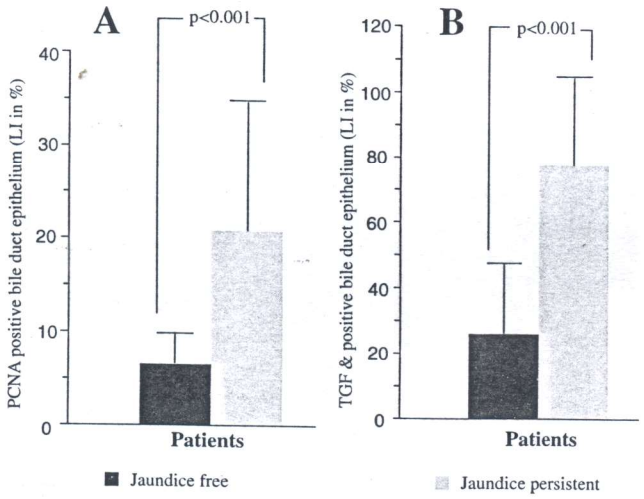


Fig. 3A, B. Expression of **A** proliferating cell nuclear antigen (PCNA) and **B** trransforming growth factor αlabeling indices (LI) in intrahepatic bile-duct epithelium of biliary atresia patients in relation of clinical outcome.

The LIs of PCNA and TGFα were not related to patient age at the time of hepatic portoenterostomy in the two groups divided at the age of 60 days. In the patients operated upon before and after 60 days of age, the expression of PCNA was 13.1 ±11.6% and 14.4±15.3% (P <0.8) and that of TGFα 47.1±31.9% and 53.5±35.1%, respectively (P <0.6).

Discussion

Achievements in modern immunohistochemistry have opened a new era in the identification of various cellular proteins in both neoplastic and non-neoplastic conditions.[3, 6, 17, 18, 20] PCNA and TGFα are detected in proliferating cells because of their high concentrations during the proliferating state.[3, 5, 14, 15] We have studied them in portal bile-duct epithelium in patients with BA by applying monoclonal anti-PCNA and -TGFα are expressed at a high frequency in intrahepatic portal bile-duct epithelium in BA: they were expressed in 100% of the cases of BA examined. PCNA staining was restricted to the nucleus and TGFα to the cytoplasm and cell membrane of biliary epithelial cells. The study confirms that proliferation of portal bile-duct epithelium has a positive correlation with clinical outcome in BA patients.

Our previous studies of the intrahepatic bile ducts using light and electron microscopy disclosed that the bile ducts are pathological even in the early stages of BA.[8, 9] Other histologic studies have also indicated that bile-duct injuries in the form of inflammation, degeneration, and obstruction are the usual features of BA.[2] A proliferative response of the biliary epithelium in BA would most likely take place as a regenerative process to repair injured bile ducts of any etiology. However, factors that determine the proliferative response of biliary epithelial cells are not yet clearly understood. The fact that there

were variations in the expression of proliferative activity in individual patients can be explained by the concept that a proliferative response may depend partly on the severity of previous damage and the differing abilities of individual patients to repair damage. The present study confirms for the first time using immunohistochemical methods that the epithelial response of bile ducts and ductules is proliferative rather than degenerative in BA.

The pathology of the intrahepatic bile ducts is regarded as one of the most important factors determining the prognosis of BA.[8,9] The present study demonstrates that patients with persisting jaundice have significantly higher proliferating indices of PCNA and TGFα expression in the bile-duct epithelium than patients who are jaundice-free during their postoperative course. The intimate relationship between the regenerating response of bile-duct epithelium and clinical outcome may indicate that less prominent proliferation of the biliary epithelial cells in jaundice-free patients is the result of a lesser degree of injury to the portal bile-duct epithelium. In contrast, the prominent regenerative response of the biliary epithelial cells in the group with persistent jaundice may be due to more severe bile-ducts injury from unknown aetiologic agents or to severe, long-standing obstruction. Our previous study of PCNA expression in the hepatocytes in BA also disclosed a similar finding: PCNA expression in the hepatocytes of jaundice-free patients was significantly lower than in those of jaundiced patients.16

This study also reveals that the expression of PCNA-immunoreactive epithelial cells is more prominent in peripheral than in central bile ducts in the portal area. It would be interesting to know why the structures near the hepatic lobules (peripheral portal tract) appeared more regenerative. The damaging effects of the unknown aetiologic agents seemed much more severe in the periphery adjacent to the hepatic lobules than in the centre of the portal tract. Nostrant *et al* found that rat liver-cell regeneration after selective zonal injury was directly related to the site of damage of a particular area in the liver, and concluded that the contribution of different acinar zones to regeneration after toxic liver injury depends upon the distribution of damage within the acinus.[21]

In conclusion, PCNA and TGFα expression in the epithelial bile ducts of the portal tract could be a good marker to assess the overall condition of the bile ducts, and thereby serve as a useful parameter to predict the clinical outcome of BA patients.

References

1. Kobayashi A, Itabashi F, Ohbe Y. 1984. Long term prognosis in biliary atresia after hepatic portoenterostomy: analysis of 35 patients who survived beyond 5 years of age. *J Pediatr* 105: 243-246
2. Bill AH, Haas JE, Foster GL. 1977. Biliary atresia: histopathologic observations and reflections upon its natural history. *J Pediatr Sugr* 12: 977-982
3. Castella A. Prieto J, Fausto N. 1991. Transforming growth factor β 1 in chronic liver disease: effect of interferon α therapy. *N Engl J Med* 324:933-940
4. Bravo R, Frank R, Blundell PA, Madonald-Bravo H. 1987. Cyclin/PCNA is the auxiliary protein of DNA polymerase delta. *Nature* 326: 515-517
5. Bravo R, Macdonald-Bravo H. 1987. Existence of two populations of cyclin/proliferating cell nuclear antigen during the cell cycle: association with DNA replication sites. *J Cell Biol* 105: 1549-1554
6. Robins BA, Vega DL, Ogata K, Tan EM, Nakamura RM. 1987. Immunohistochemical detection of proliferating cell nuclear antigen in solid human malignancies. *Arch Pathol Lab Med* 111: 841-845
7. Celis JE, Bravo R, Larsen PM Fey SJ. 1984. Cyclin: a nuclear protein whose label correlates directly with the proliferative state of normal as well as transformed cells. *Leuk Res* 8: 143-157
8. Ito T, Horisawa T, Ando H. 1983. Intrahepatic bile ducts in biliary atresia - a possible factor determining the prognosis. *J Pediatr Surg* 18:124-130
9. Ito T, Iio K, Iyomasa Y. 1983. Electron microscopic studies of livers of biliary atresia. In: Kasai M (ed) Biliary atresia and its related disorders. *Excerpta Medica*, International Series 627, pp 88-95
10. Lilly JR, Karrer FM, Hall RJ, Stelling GP, Stevez JE, Greenholz SK, Wanek EA, Schroter GPJ. 1989. The surgery of biliary atresia. *Ann Surg* 210:289-296
11. Kasai M. 1983. Advances in treatment of biliary atresia. *Jpn J Surg* 13: 265-276
12. Kasai M, Mochizuki I, Ohkohchi N, Chiba T, Ohi R.1989. Surgical limitation for biliary atresia: indication for liver transplantation. *J Pediatr Surg* 24:851-854
13. Gautier M, Valayer J, Odievre M, Alagille D. 1984. Histopathological liver evaluation 5 years after surgery for extra hepatic biliary atresia: a study of 20 cases. *J Pediatr Surg* 19:263-268
14. Miyachi K, Fritzler MJ, Tan EM. 1978. Auto antibody to nuclear antigen in proliferating cells. *J Immunol* 121: 2228-2234
15. Sporn MB, Roberts AB. 1985. Autocrine growth factors and cancer. *Nature* 313:745-747
16. Hossain M, Murahashi O, Ando H, Iio K, Kaneko K, Ito T. 1995. Immunohistochemical study of proliferating cell unclear antigen (PCN/cyclin) in hepatocytes of biliary atresia - a useful parameter to predict clinical outcome. *J Pediatr Surg* 30: 1297-1301
17. Kawakita N, Seki S, Yanai A, Sakaguchi H, Kuroki T, Mizoguchi Y, Kobayashi K, Monna T. 1991. Immunocytochemical identification of proliferative hepatocytes using monoclonal antibody to proliferating cell nuclear antigen (PCN/cyclin), comparison with immunocytochemical staining for DNA polymerase-alpha. *Am Clin Pathol* 97:14-20
18. Fausto N, Mead JE. 1989. Regulation of liver growth: protooncogenes and transforming growth factors. *Lab Invest* 60:4-13
19. Derynck R. 1988. Transforming growth factor α . *Cell* 54: 593-595
20. Castellani R, Visscher DW, Wykes S, Sarkar FH, Crissman JD. 1994. Interaction of transforming growth factor-alpha and epidermal growth factor receptor in breast carcinoma - an immunohistologic study. *Cancer* 73:344-349
21. Nostrant TT, Miller DL, Appelman HD, Gumucio JJ. 1978. Acinar distribution of liver cell regeneration after selective zonal injury in the rat. *Gastroenterology* 75:181-186

SELF assessment

SURGERY

1. In an uncomplicated dislocation of the glenohumeral joint, the humeral head usually dislocates in which of the following directions?
   A. Anteriorly or anteroinferiorly
   B. Superiorly or posterosuperiorly
   C. Directly posteriorly
   D. Laterally
   E. None of the above

2. A multinodular mass from the lower inner quadrant of a 45-year old woman's breast is biopsied. The pathologic diagnosis is cystosarcoma phylloides. Which of the following surgical procedures should be performed?
   A. Radical mastectomy
   B. Modified radical mastectomy
   C. " + internal mammary node dissection
   D. Simple mastectomy
   E. "Lumpectomy"

3. Peritonitis is usually an indication for surgery. It is most commonly the result of :
   A. A perforated peptic ulcer
   B. A ruptured appendix
   C. A ruptured diverticulum
   D. A gangrenous bowel
   E. Emphysematous cholecystitis

4. Marked reduction in uterine muscle tone is a consequence of which of the following anaesthetics?
   A. Ether
   B. Halothane
   C. Nitrous oxide
   D. Thiobarbiturate
   E. Intrathecal procaine

ENDOCRINOLOGY

1. Which of the following thyroid tumours is known to produce calcitonin?
   A. Follicular adenoma
   B. Medullary carcinoma
   C. Papillary carcinoma
   D. Follicular carcinoma
   E. Papillary adenoma

2. The three most frequent manifestations of primary aldosteronism are :
   A. High renin level, hypertension and stroke
   B. Hypertension, hyperkalaemia and muscular weakness.
   C. Hypertension, hypokalaemia and tetany
   D. Polyuria, polydipsia and polyphagia
   E. Hypokalaemia, polydipsia and hypertension

3. Lymphadenoid goitre is more commonly known as :
   A. Woody thyroid
   B. Hashimoto's disease
   C. Grave's disease
   D. De Quervain's thyroiditis
   E. Multinodular goitre

4. Which of the following findings would indicate a diagnosis of adrenal cortical tumour rather than abnormal hyperplasia?
   A. 17-ketosteroid excretion is suppressed by dexamethasone
   B. ACTH administration results in increased 17-ketosteroid secretion
   C. There is failure of suppression of 17-ketosteroid by dexamethasone
   D. There is high level of plasma testosterone
   E. 17-ketosteroid excretion in 24 hours is less than 15 mg

ANSWERS:

Surgery:

1. A 2. D 3. A 4. A

Endocrinology

1. B 2. E 3. B 4. A

TWO MINUTES WITH DIABETES

THE PROBLEM

"Why am I so swollen?"

THE CASE

Ms. Lisa is a 21 years old student with diabetes of 15 years duration. During a stormy adolescent period, she experienced several episodes of ketoacidosis. Now she has the residuals of vasoproliferative retinopathy, which followed successful treatment by bilateral laser photocoagulation. Her eye grounds show fibroglyosis: her visual acuity was good. Her blood pressure is normal. She retains no fluid under ordinary circumstances but does show a trace to 1+ albumin in the urine. Her serum creatinine is normal.

Recently Ms. Lisa developed ketoacidosis following a bout of nausea, vomiting and diarrhoea. Her acidosis was corrected and her diabetes re-regulated by usual, low dose insulin regimen. Four days later she noted swelling of her legs. One week later she had gained 12 pounds. There was pitting oedema of both legs, a swollen abdomen with increase in abdominal girth, and tenderness of the liver. Both periorbital areas were puffy. She wanted to know why she was so swollen.

DISCUSSION

Her kidney function was unchanged with only small amount of albumin in the urine. The serum creatinine remained normal; the serum proteins were normal.

The timing and nature of her problem suggests the diagnosis of "Insulin oedema." Since insulin has been available, catabolic diabetics who are treated vigorously with it have noted significant weight gain even before good control of hyperglycaemia has been established. Such patients also report a decrease in urine volume. Studies have suggested that insulin has an antinatriuretic effect. Insulin oedema is identified in insulin dependent diabetic patients who have been in poor metabolic control for prolonged period, or who are recovering from ketoacidosis. A rapid rise in weight is followed by extremity oedema and occasionally is associated with ascitis and hepatomegaly. These patients have increased glycogen stores in the liver and retained water. Insulin oedema usually lasts six to eight weeks and is treated with salt restriction, diuretics and reassurance; it is self limiting in nature. Ms. Lisa was given Frusemide and salt restriction. She lost 16 pounds over the ensuing four weeks. Eight weeks after ketoacidosis she weighted 121 pounds - her usual weight.

POINTS TO REMEMBER

When a patient has severe oedema following treatment for acute decompensation of diabetes, consider "Insulin oedema", a self-limiting disorder. ■

(Continued from page no. 14)

OPERATIVE TREATMENT

1. Closed reduction } Evaluation of reduction → Internal fixation (AO
2. Open reduction } screws, Knowle's pins, DHS)
3. Hemiarthroplasty
4. Excision arthroplasty (Girdlestone)
5. Total hip replacement.

The definite management plan has to be individualized. A schematic presentation of options has been proposed (flow chart *page 14*)

COMPLICATION OF AN IMPACTED FRACTURE TREATED NONOPERATIVELY

1. Nonunion
2. Aseptic necrosis: 13-44% and
3. General complications e.g., Pulmonary.

COMPLICATIONS

In Children:

1. Avascular necrosis
2. Coxa vera
3. Nonunion
4. Premature closure of epiphysis

In Adults:

1. Avascular necrosis
2. Nonunion
3. Malunion/Coxa vera/Coxa valga
4. Thrombo-embolism
5. Infection

CONCLUSION

A concise description of the femoral neck fractures covering most areas of it is difficult. The attempt here is a reminder of known facts from a single article that would otherwise make one browse many such articles. Conclusively, femoral neck fractures have to be individualized in terms of the type, extent of displacement, patient's age, activity level and physical condition as well as functional demand expected. For the same, one has to be familiar with its anatomic aspects and the likely outcome. This understanding has successfully reduced morbidity and mortality of the patients with fracture neck of femur. ■

MOTHER NEEDS ZINC for normal embryonic development even during lactation for normal growth of the Infant

Dr Md Javed Sobhan

Recent research and technological development in medical science is updating and upgrading the knowledge of professionals about the importance of Zinc therapy like other essential elements of the body. Maternal nutritional status, with respect to both macro- and micronutrients, is known to be an important determinant of perinatal and neonatal survival and well-being; but maternal nutrition programs in developing countries have focused almost exclusively on the risks to the mother and infant from maternal iron deficiency anaemia during pregnancy. Other micronutrients such as Zinc may also be important for maternal and infant survival during the critical period surrounding birth, but their importance is less well understood.

Zinc is an essential component of many enzymes including carbonic anhydrase, alkaline phosphatase, lactic dehydrogenase and appears to help stabilize RNA and DNA and to be part of several transcription factors. Zinc supplementation reduces the incidence of childhood infections and may lower child mortality. Moreover Zinc is essential for the normal embryonic development. Systems influenced by Zinc include: Reproductive System, Immune System, Neurologic System, Dermatological System, Gastro-intestinal System.

These functions indicate the importance of maintaining proper Zinc level in our body. The blood plasma Zinc concentration is approximately 100µg/100ml, 70% of which is bound to albumin and most of the rest is associated with α_2 macroglobulin, although a small amount is bound to uncharacterised proteins.

Normal daily requirement: The daily requirement varies with age and growth state. The approximate requirements are:

a)
1. At one month of age - 800µg/day
2. Between 1-10 years - 3-10mg/day
3. In normal adults - 10-15mg/day
4. During pregnancy - 20-25mg/day

b)
1. Birth to six months - 0-3mg/day
2. Six months to 1 year - 5mg/day
3. 1 year to 10 year - 10 mg/day
4. Over 10 years - 15 mg/day

Dosage range for treating deficiency: It depends on the individual and on the cause and severity of the deficiency. In general, 30-50 mg daily, usually required in the form of Zinc sulphate.

Causes for negative Zinc balance (Zinc deficiency): Intestinal diseases, Anorexia, Use of contraceptives, Haemodialysis, Burns, Chronic alcoholism, Diabetes mellitus, Nephrotic syndrome, Chronic febrile illness.

Dietary Sources of Zinc: Oysters, wheat bran, liver, beef, other meats, whole meal wheat flour and bread, oatmeal, sardines, crab, nuts, breakfast cereals.

Absorption: Absorption of Zinc in small intestine is decreased by fibres, phytate, calcium and copper and increased by glucose, amino acids, peptides, iodoquinolone and other chelating agents.

Excretion: About 2 to 5 mg of Zinc is excreted each day through secretion of pancreas and intestine. Losses also occur through the renal proximal tubules.

Drug interactions: Large amount of Calcium decreases the absorption of Zinc. A conditioned deficiency of copper including anaemia is caused by Zinc supplement in excess of 10 (ten) times the recommended daily allowance.

Toxicity: Toxicity follows inhalation of Zinc fumes (by welders), oral ingestion, or intravenous administration. It is manifested by fever, chills, excessive salivation, headache, cough, and leucocytosis, anaemia, and central nervous system disturbances.

ZINC DEFICIENCY MAY CAUSE FOLLOWING DISORDERS

- **Growth retardation:** Zinc deficient cells fail to divide and differentiate, with consequent growth impairment.
- **Impaired spermatogenesis:** Testicular Zinc is critical for normal spermatogenesis and for sperm physiology; it preserves genomic integrity in the sperm and stabilizes attachment of sperm head to tail.

Dr Md Javed Sobhan, MBBS, Executive, MSD

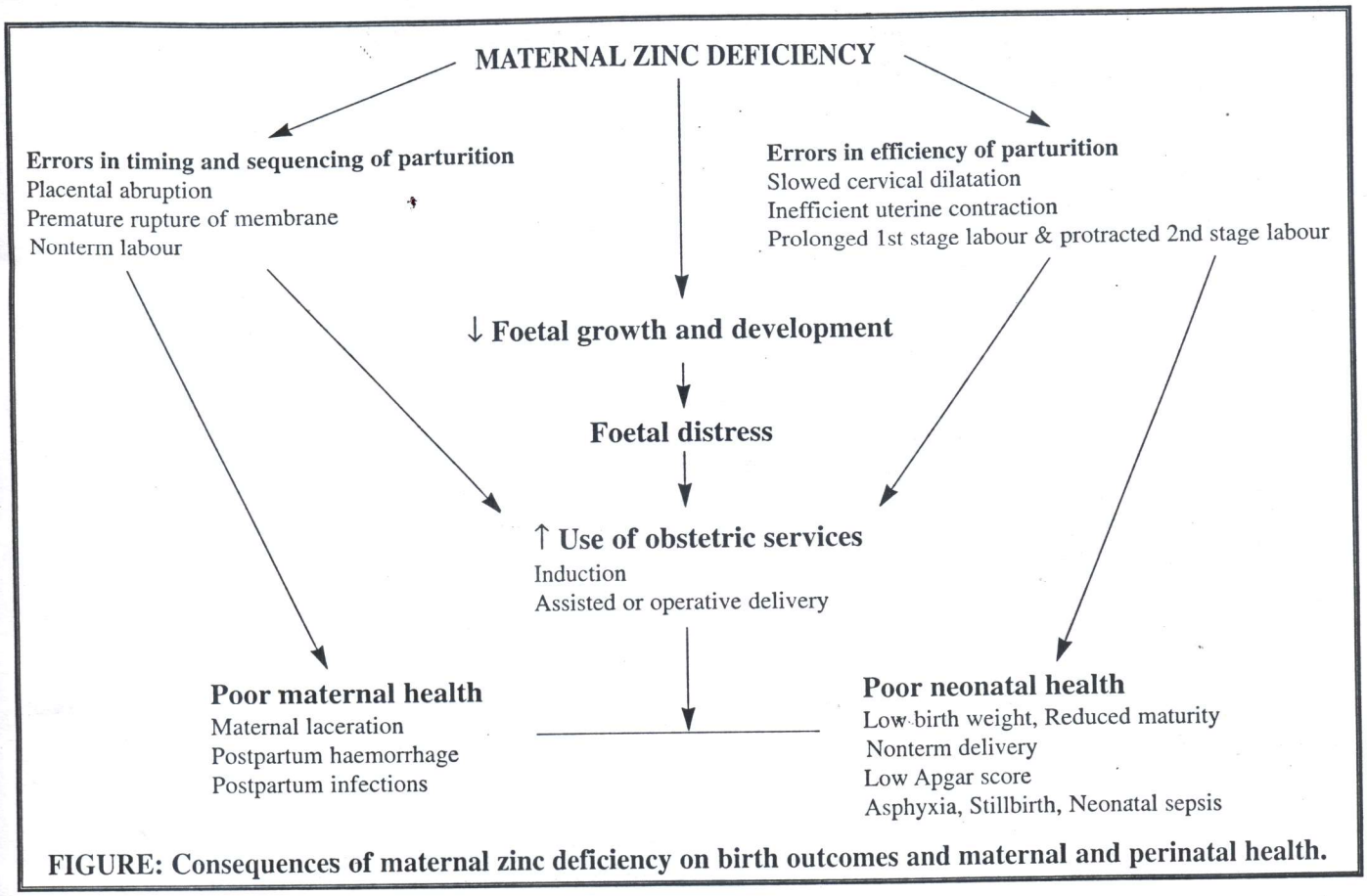


FIGURE: Consequences of maternal zinc deficiency on birth outcomes and maternal and perinatal health.

• **Congenital malformation:** Zinc is essential for normal embryonic development. Deficiency results in malformations of the brain, eyes, bones, heart, and other organs. The survival of embryo is placed at risk when Zinc intake is reduced even for a period of days particularly in the first trimester.

• **Immunological dysfunction:** Zinc is essential for the formation and function of the immune system. With Zinc deprivation, the thymus atrophies and viable thymocytes are not formed. The function of macrophages and T-cells are impaired. The result is an inability to respond to antingens or defend the organism against infections.

• **Respiratory tract infections:** In a recent study, it has been shown that Zinc deficiency increases the incidence of respiratory tract infections in children. It has been also shown that Zinc supplementation reduces the prevalence of cough and respiratory tract infection by 45%.

• **Dermatological dysfunctions:** Zinc deficiency may cause dermatological dysfunctions including hyperkeratosis, parakeratosis, acrodermatitis and alopecia.

• **Diarrhoea:** Zinc deficiency increases the incidence of diarrhoea. Supplementation of Zinc reduces the prevalence of diarrhoea by 25 to 30%

Conclusion

Some clinical studies show that there is a decline in plasma Zinc during the 1st trimester, which continues throughout pregnancy, even in the presence of optimal zinc in food and the requirement for Zinc during lactation is greater than those during pregnancy, especially during the early weeks of postpartum. They also show that Zinc supplementation has been associated with better motor development in very low birth weight infants and has been associated with better neuropsychologic functioning in first grade students.

Taking into account of the impact of Zinc therapy in maternal health and infant development, doctors community especially gynaecologists and obstetricians may consider the use of Zinc in prescribing their patients. At the same time Government and non-governmental organisations (NGOs) may consider working out programmes similar to Iodine therapy, vitamin A therapy, EPI etc. to create mass awareness about Zinc therapy. ■

References:

1. *Harrison's* 14th ed. (p. 490-491)
2. *Davidson's* 17th ed. (p. 561)
3. *BMJ* 8th August 1998
4. *American Journal of Clinical Nutrition* Vol. 66 & 68, 1998

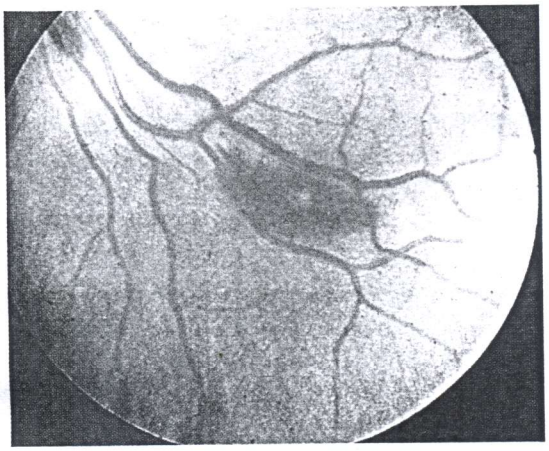
Fevac

Dependable and Safe

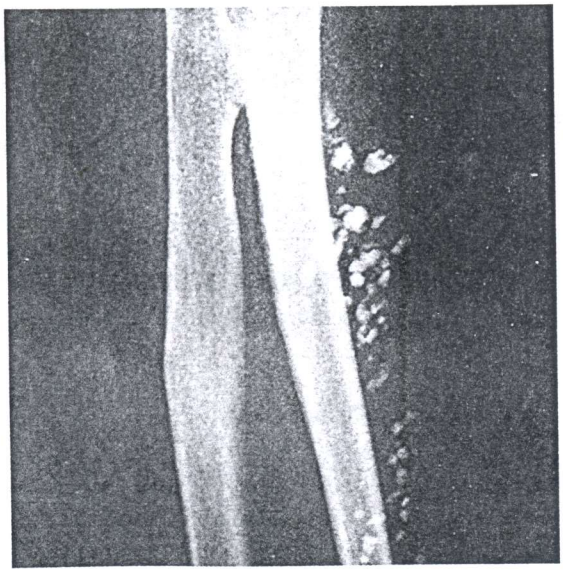
Fevac

Paracetamol (Tablet/Suspension)

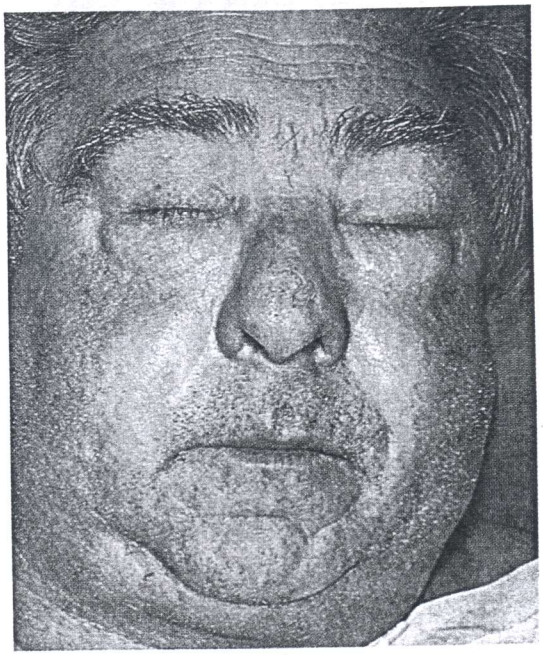
MEDI QUIZ 2



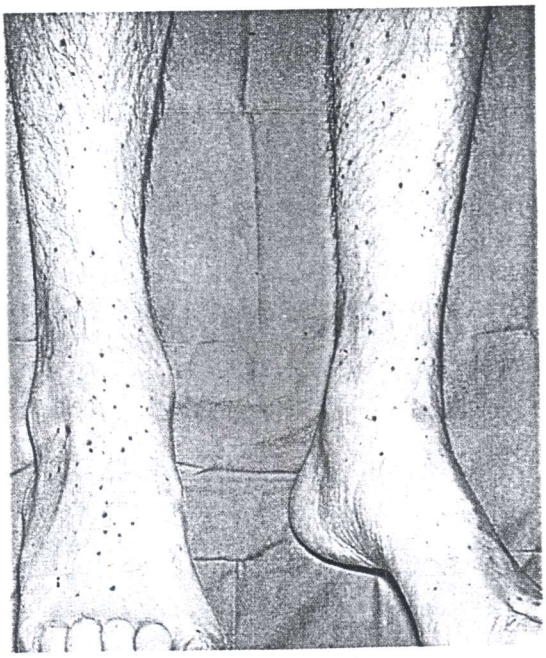
1. a) What is the name of this retinal lesion?
   b) Name the condition(s) in which the lesion is seen.



2. a) What lesion is seen in this radiograph of forearm?
   b) What is the diagnosis?



3. This patient becomes acutely dyspnoeic shortly after a diagnostic procedure carried out in a general medical ward.
   a) What name is given to the appearance seen here?
   b) Which diagnostic procedure was performed?



4. This patient's blood film is abnormal.
   a) What abnormality should be sought on abdominal examination?
   b) What diagnostic value would this abnormality have?

Please Use the Answer Sheet Opposite Page No. 28
Answers to MediQuiz 2 must reach us within ***31st March 1999.***

DRUG induced side effects

RESPIRATORY SYSTEM

Airway obstruction (Bronchospasm; Asthma)

Adenosine
Beta blockers
Cephalosporins
Cholinergic drugs
NSAIDS, e.g. aspirin, indomethacin
Penicillins
Pentazocine
Streptomycin
Tartrazine (drugs with yellow dye)

Cough

ACE inhibitors

Nasal Congestion

Decongestant abuse
Guanethidine
Isoproterenol
Oral contraceptives
Reserpine

Pulmonary Oedema

Contrast media
Heroin
Hydrochlorthiazide
Interleukin 2
Methadone

Pulmonary hypertension

Fenfluramine

Pulmonary infiltrates

Acyclovir
Amiodarone
Bleomycin
Busulphan
Carmustine (BCNU)
Chlorambucil
Cyclophosphamide
Gold
Melphalan
Methotrexate
Methysergide
Mitomycin C
Nitrofurantoin
Procarbazine
Sulphonamide

Respiratory depression

Aminoglycosides
Hypnotics
Opiates
Polymyxins
Sedatives
Trimethaphan

MUSCULOSKELETAL SYSTEM

BONE DISORDERS

Osteoporosis

Glucocorticoid
Heparin

Hyperuricaemia/Gout

Aspirin (Low dose)
Chlorthalidone
Cyclosporine
Cytotoxics
Ethacrynic acid
Fructose (I.V.)
Frusemide
Hyperalimentation
Pyrazinamide
Thiazide

Osteomalacia

Aluminium hydroxide
Anticonvulsants
Glutethimide

MYOPATHY or MYALGIA

Amphotericin B
Carbenoxolone
Chloroquine
Cimetidine
Clofibrate
Glucocorticoid
Oral contraceptive
Zidovudine

RHABDOMYOLYSIS

Gemfibrozil
Lovastatin

Medi News

Zinc cuts childhood respiratory infections

A study done in India has shown that zinc supplements cut the incidence of acute lower-respiratory infection (ALRI) in young children by as much as 45%.

Sunil Sazawal and co-workers from the All India Institute of Medical Sciences in New Delhi and Johns Hopkins School of Public Health (Baltimore, MD, USA) randomly assigned 609 children to receive either a daily multivitamin supplement or a multivitamin supplement plus elemental zinc 10mg for 6 months (*Pediatrics* 1998; 102: 1-5). Field workers administered supplements daily while another team visited the families every 5 days to ask if the children had fever, cough, or difficulty of breathing in the preceding 5 days. Children with raised respiratory rates were treated with antibiotics.

24 episodes of ALRI occurred in the zinc group compared with 44 episodes in the control group, a 45% reduction (odds ratio 0.55, 95% CI 0.33-0.90; p=0.02). These findings, plus other known benefits of zinc on immunity, growth etc. "indicate that interventions to improve zinc intake deserve more attention as means to improve child health", they conclude.

TB Gene Code Revealed

What has 4000 genes, more than 4 million base pairs, and a high guanine and cytosine content?

The genetic code of the world's most deadly infectious organism, *Mycobacterium tuberculosis*, as reported by an international team of scientists in the June 11 issue of *Nature*. With 4 411 529 base pairs, the *M tuberculosis* genome is the second-largest bacterial genome ever deciphered, behind that of *Escherichia coli*. The research team, composed of scientists from the United Kingdom, France, Denmark, and the United States, sequenced the genome of the H37Rv strain of the tuberculosis (TB) bacterium, a commonly studied variety that is not highly infectious.

Among the new discoveries they have revealed about the centuries-old bacterium is that its genome contains a large number of repeating sequences of base pairs that apparently code for proteins that make up the outer coat of *M tuberculosis*. The result seems to be a regularly changing protein coat that makes the bacterium capable of dodging attacks by the immune system.

According to the World Health Organization, TB claims 3 million lives annually worldwide. Experts hope that with the genome sequenced the pace of new drug and vaccine development will accelerate.

Genetic Family Affair

Initially considered an orphan gene without relatives, the *p53* tumor suppressor gene is not such a loner after all according to new findings published in *Nature Medicine* in July '98.

Researchers in Japan report that they have cloned the *p51* gene, which is structurally and functionally similar to apoptosis-inducing *p53*. Mutated *p53* is believed to contribute to the development of about half of all human cancers. At the same time, a team from Johns Hopkins University School of Medicine has identified another *p53* homologue, which they call *p40*. Their research indicates that *p40* is the human homologue of the rat *ket* gene. Last year, the Hopkins team identified the first *p53* homologue, known as *p73*.

Unlike the ubiquitously expressed *p53*, the *p51* gene, said the Japanese team, is expressed in limited types of tissue, including skeletal muscle, placenta, mammary gland, prostate, heart, and lung. They found that *p51* also induces apoptosis and that mutated versions are present in some human epidermal tumors.

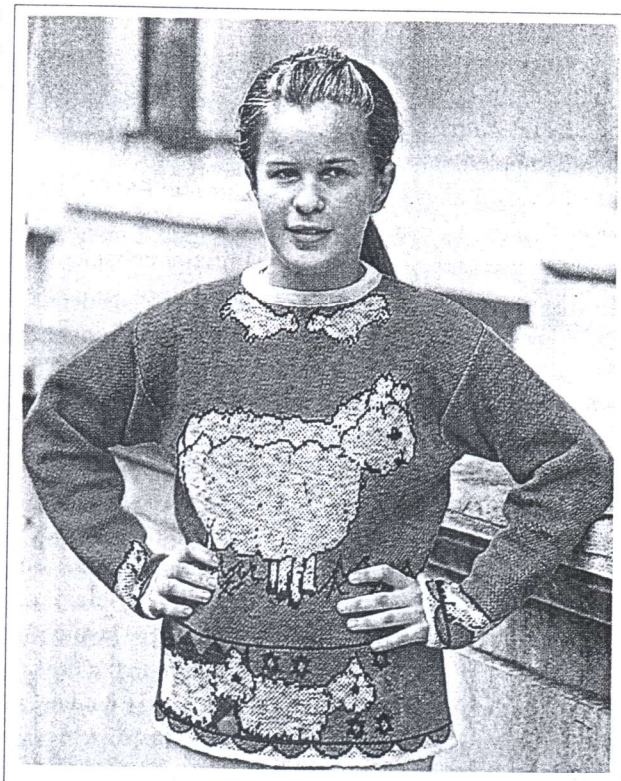
The findings have important therapeutic potential, the Japanese researchers said, since manipulation of the homologues in tumours with mutated *p53* may be able to keep cells from becoming cancerous.

New Cancer Markers

Physicians treating patients with head and neck cancer may have new clinical options for predicting recurrences and staging treatment.

Researchers at the University of Pittsburgh School of Medicine report that levels of epidermal growth factors receptor (EGFR) and its ligand, transforming growth factor α (TGF-α), can predict clinical outcomes as accurately as the traditional method of cervical lymph node dissection. Their finding is based on examinations of tumour tissue taken from 91 patients with head and neck cancer, which they compared with previous studies of patients without the disease. The research team used monoclonal antibodies and computerized image analysis to measure levels of EGFR and TGF-α.

In their report, published June 3 '98 in the *Journal of the National Cancer Institute*, the researchers said patients with head and neck cancer had elevated levels of the proteins in the tumour tissue, while patients without the disease had low levels. "We could use this information to identify patients at risk for recurrent disease and develop a targeted therapy based on the biology of these markers," said Jennifer Rubin Grandis, MD, lead author of the study.



A sweater made from wool of Dolly, the cloned sheep, was put on display at the Science Museum in London. Holly Wharton, aged 13, designed the sweater as part of a national competition run by the museum, Portman Building Society, and the Cystic Fibrosis Trust, London, UK.

Lamivudine promising in chronic hepatitis B

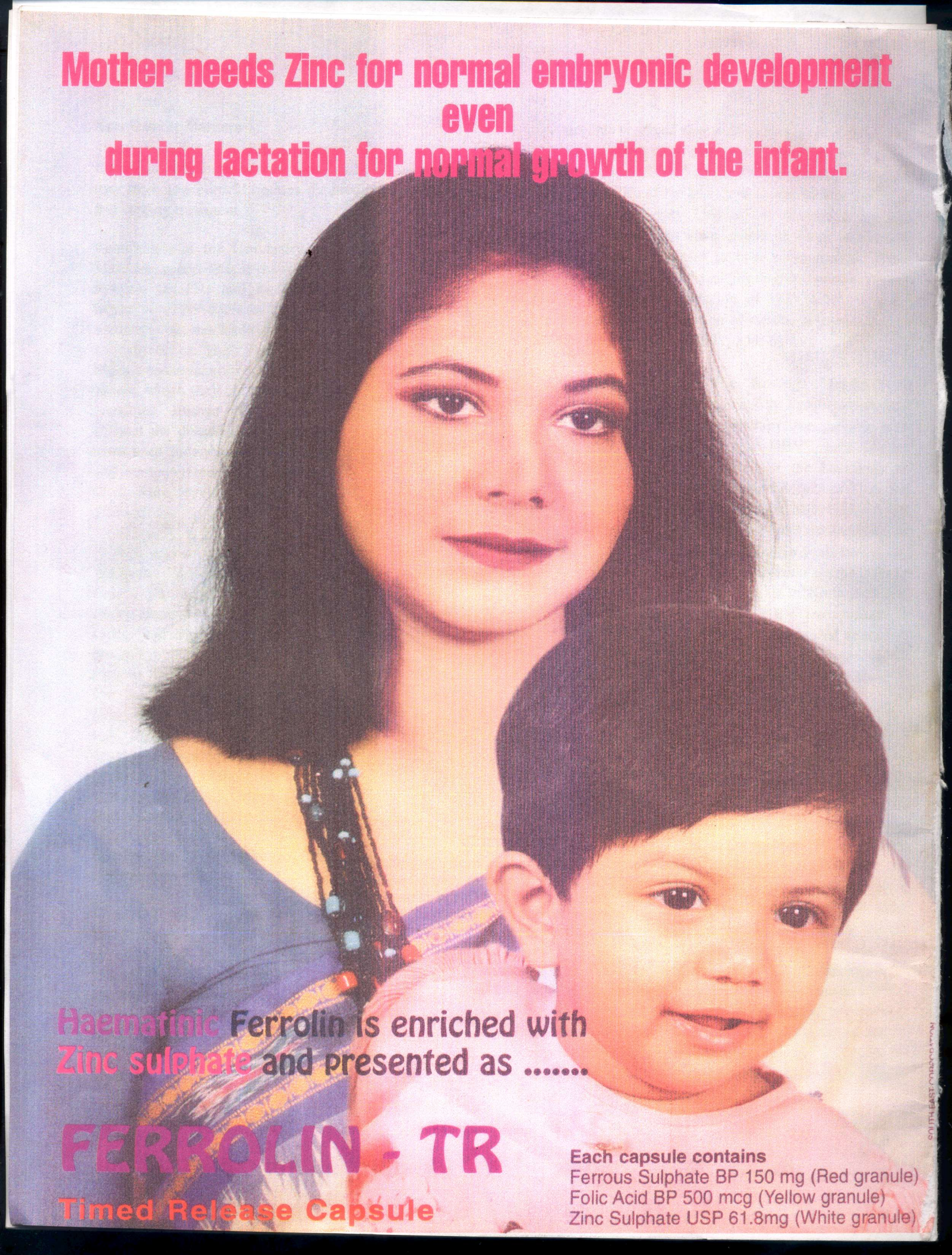
Treatment with the oral antiviral agent lamivudine (3TC) results in a "substantial" reduction in liver inflammation and fibrosis, and a significant increase in seroconversion rates, in patients with chronic hepatitis-B-virus (HBV) infection, say investigators for the Asia Hepatitis Lamivudine Study Group. These results are important because the response rate to interferon-α, the approved HBV therapy, is low in Asia where the disease is most prevalent.

142 patients received lamivudine 25 mg once daily; 143 received 100 mg once daily; and 73 patients were given placebo. All had liver biopsies before and on completion of the trial. 49% of the low-dose group, 56% of the high-dose group, but only 25% of the placebo group had substantial reductions (two points or more in Knodell necroinflammatory score) in liver inflammation. Those taking 100 mg of drug had the highest seroconversion rate, the greatest suppression of HBV DNA, and the highest rate of normalisation of alanine aminotransferase activity (*N Engl J Med* 1998; 339: 61-68).

The findings "represent a potentially significant advance in therapy for patients with chronic HBV", says Charles Howell of the University of Maryland School of Medicine (Baltimore, MD, USA). A US team reported similar results at a meeting in May, he recalls, "so lamivudine seems to be as efficacious in North America, where HBV transmission is primarily by sexual contact or intravenous drug use, as in Asia, where transmission is mainly vertical".

In an editorial, Masao Omata (University of Tokyo, Japan) notes that lamivudine suppresses viral replication but "does not eliminate the source of the replication", so the virus may flare up again after the drug is stopped. If the liver biopsies had been done some weeks after the trial, he writes, the percentage of patients with improved necroinflammatory activity "might have been much lower". Howell agrees, noting that "questions remain" about the drug's impact on the long-term natural history of HBV, optimisation of treatment, and whether monotherapy will "ultimately be acceptable" given that about 14% of patients developed drug-resistant viral strains.

Mother needs Zinc for normal embryonic development even during lactation for normal growth of the infant.



Haematinic Ferrolin is enriched with Zinc sulphate and presented as .....

FERROLIN - TR

Timed Release Capsule

Each capsule contains
Ferrous Sulphate BP 150 mg (Red granule)
Folic Acid BP 500 mcg (Yellow granule)
Zinc Sulphate USP 61.8mg (White granule)

SOUTH EAST CORPORATION