पल्स Pulse

खंड 12
Volume 12

मार्च 2010
March 2010

अंशदायी स्वास्थ्य सेवा स्कीम
CONTRIBUTORY HEALTH SERVICES SCHEME

भारत सरकार
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पल्स
PULSE
BARC Hospital Newsletter
खंड 12 मार्च 2010
Volume 12 March 2010

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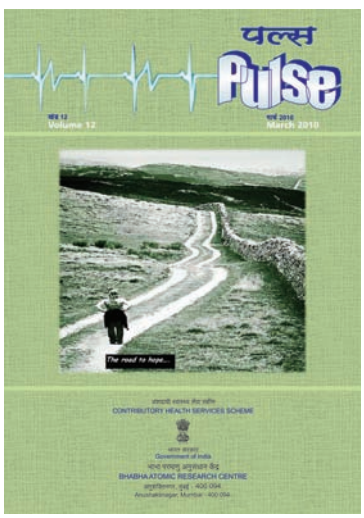

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CONTENTS

From the Editor's Desk

The 'War on Cancer' rages on. There is still no cure for most advanced stage malignancies. Even after the treatment is complete, one does not know what to expect. There is a feeling of mixed emotions and there are concerns that cancer may resurface any time. The patients need counselling from time to time even if they had early stage cancers at the time of their treatment.

The impact of major breakthroughs in cancer treatment, in terms of Gene Therapy is felt only in research laboratories. Gene Therapy is still in its infancy. Repairing of all gene mutations in cancer is the biggest challenge. There are efforts to develop 'smart bombs' that will target only the cancer cells and will not cause damage to the normal cells. One of the approaches is to deliver genes into cells by viral vectors and direct DNA injections. Attempts are also being made to change the genetic code of cancer cells in the body. But it seems unlikely that gene therapy will provide this kind of magic treatment for cancer in the near future.

Combination of various treatments is necessary for treating cancer. The cancer cells utilize multiple pathways for their growth, the inhibition of one pathway leads to the utilization of another as a compensatory mechanism. Targeted therapies are a new class of cancer therapeutic agents, with specific action on these pathways. Traditional chemotherapy may have to be combined with targeted therapy, for the treatment of metastatic cancers. This particular strategy can at least improve the quality of life and can prolong life in cases of metastatic cancers. Treatment of Cancer is such a complex problem that any gain will only be incremental. The Guest Article on "Targeted therapies in Cancer" gives a detailed overview about the mechanism of action of these drugs and future directions.

Still a lot of work remains to be done for the complete cure of cancer. A combination of knowledge, research and technology may lead to the success in its treatment.

Dr. Amrita Misri

"The only ones amongst us who will be really happy are those who will have sought and found how to serve."

- Albert Schweitzer

Introduction to Targeted Therapies in Cancer and Review of Monoclonal Antibodies

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SUMMARY

Targeted therapies are a new class of cancer therapeutic agents, that have more specific action on pathways uniquely present or upregulated in cancer cells. Monoclonal antibodies (Mabs) are a type of targeted therapy, whose structure is comprised of proteins in the immunoglobulin family, that bind to specific protein epitope targets on cancer and stromal cells, allowing them to be successfully exploited as therapeutic agents. The prototype Mabs were produced from fusion of mouse B lymphocytes and mouse myeloma cells and were entirely murine in sequence. Subsequent advances in technology have allowed for humanized Mabs, which have different pharmacokinetic properties than murine Mabs in humans. Antitumour activity is mediated through direct interaction with specific target molecules, deployment of immune cytotoxic pathways, or through chaperoning cytotoxic agents to tumour. Mabs are typically administered intravenously, are generally well tolerated and can have powerful anticancer activity. Humanized Mabs have a half life in human sera of 2–3 weeks, which determine the frequency of administration. Monoclonal antibodies are currently used as standard of care, as first and second line therapy for a number of hematological malignancies and solid tumours.

Key Words – Targeted therapy, Monoclonal antibody, Chemo immunotherapy.

I. Introduction to Targeted Therapies

Over the past few decades, systemic therapy of cancers has greatly relied on a class of drugs known as 'cytotoxics' or 'chemotherapy'. The use of these drugs has significantly improved the outcome in many human malignancies including solid tumours and hematological cancers. As a class, chemotherapeutic drugs rely on inhibition of nuclear DNA replication and cell proliferation for their anti-tumour activity. Because normal non-malignant cells employ many of the same mechanisms for cell proliferation and survival that are used by malignant cells, cytotoxic chemotherapy displays a striking non-selectivity in its therapeutic effects, that include many of its well known side effects.

In the past 10-15 years, a new class of therapeutics is emerging that has been broadly named 'targeted therapy'. The most important members of this therapeutic class (although by no means limited to only these) are monoclonal antibodies and small molecule tyrosine and other kinase inhibitors. These drugs have been developed, at least partly, by a process of rational identification of cancer cell specific targets. These targets or pathways are most often located in the cytoplasm or cell membrane and are probably more cancer cell specific, compared to DNA replication that is the target of classic cytotoxic chemotherapy. Based upon their mechanism of action, the majority of these agents do not induce acute cell death, and cancer cells are rather inhibited in their autonomous growth or transformed into a state of

quiescence. A related concept is that of 'oncogene addiction' or 'pathway addiction'. These terms refer to those pathways or genes, that are critical for cancer cell survival and without which the cell will die. Theoretically, if such a gene or pathway was to be specifically inhibited, it might be possible to selectively eradicate the tumour. Unfortunately most cancers don't rely critically or exclusively on one or a few pathways for survival. One classic example of such 'gene addiction' is that of chronic myeloid leukemia that is critically dependent on the product of the fusion 'Philadelphia chromosome' (the protein product known as BCR-ABL) for its survival. This explains the almost miraculous activity of the BCR-ABL tyrosine kinase inhibitors, in this disease. In most cancers however, the model is that of a web of pathways rather than one pathway that are utilized by the cell for its survival, proliferation and growth advantage. Thus inhibition of one pathway leads to cancer cell adaptation by compensatory up regulation (or down regulation if required) of the other pathways in the 'web', leading to resistance and escape from the inhibitor. Although explained rather simplistically here, these processes are incredibly complex and explain why decades of 'War on Cancer' has still not lead to cures for most advanced stage malignancies. Some of the pathways and processes that have been exploited for development of 'targeted therapies' are the following:

1. Cell surface receptor antibodies
2. Tyrosine kinase inhibitors
3. Farnesyl-tranferase inhibitors
4. Apoptosis agonists
5. Hormone agonists and antagonists
6. Anti-sense oligonucleotides
7. Anti-angiogenic agents
8. Metalloproteinase inhibitors
9. Immune system activators and modulators.

In this review, we will focus on monoclonal antibodies that target one or more of the above processes as anti-cancer therapeutic agents.

II. Monoclonal Antibodies in Cancer Therapeutics

Antibodies, though being highly specific for a particular molecule epitope, can effectively target cancer cells and thus be used for therapeutic purposes. The recognition of target specificity of antibodies led Paul Ehrlich to propose the concept of the 'magic bullet' at the beginning of the 20th century. Since that time, dramatic successes have helped propel receptor-specific monoclonal antibodies (Mabs) from the laboratory bench into the clinic.

In 1975, Kohler and Milstein introduced the hybridoma, a fusion of mouse myeloma and spleen cells, as a means of large-scale production of murine Mabs for which they were awarded the Nobel Prize. Initially, this method of murine Mab production provided antigen-specific reagents for laboratory studies and consequently clinical tools for assessment of tissue histopathology and serum markers of disease.

A. Antibody Structure and Function

Endogenous antibodies are immunoglobulin (Ig) synthesized by B lymphocytes. Each B-lymphocyte clone produces a unique and specific immunoglobulin. Antibodies have two separate functions :

(i) To bind specific antigen.
(ii) To recruit mediators of the immune system, including complement and effector cells.

Antibodies are proteins comprising four polypeptides with molecular weights between 150–900 kDa. The polypeptide chains contain two identical heavy chains (α, δ, γ, μ, and ε) and two identical light chains (λ, k) that join to form heterodimers linked by disulphide bonds to form a three-dimensional 'Y'-shaped protein. The two outstretched arms of the 'Y', known as the 'fragment antigen binding' or Fab portion, are responsible for recognizing and binding specific antigen.

The Fab is comprised of a constant region, a variable region and a hyper variable region that enable the antibody to bind to specific antigen epitope. The base of the 'Y' is known as the Fc portion, which mediates the physiological functions of the antibody such as

triggering antibody-dependent cell mediated cytotoxicity (ADCC) through Fc receptor on effector cells as well as providing the site for complement binding and complement-mediated killing [1] (Fig.1). There are five antibody classes: IgG, IgA, IgM, IgD and IgE. IgG (molecular weight 150 kDa) makes up approximately 70% of the antibody pool in humans and serve as the prototypical antibody. Therapeutic monoclonal antibodies are typically of the IgG type. IgG antibodies can then be divided into four subclasses, IgG1-IgG4. IgG1-IgG3 is the most active in antibody-dependent cellular toxicity.

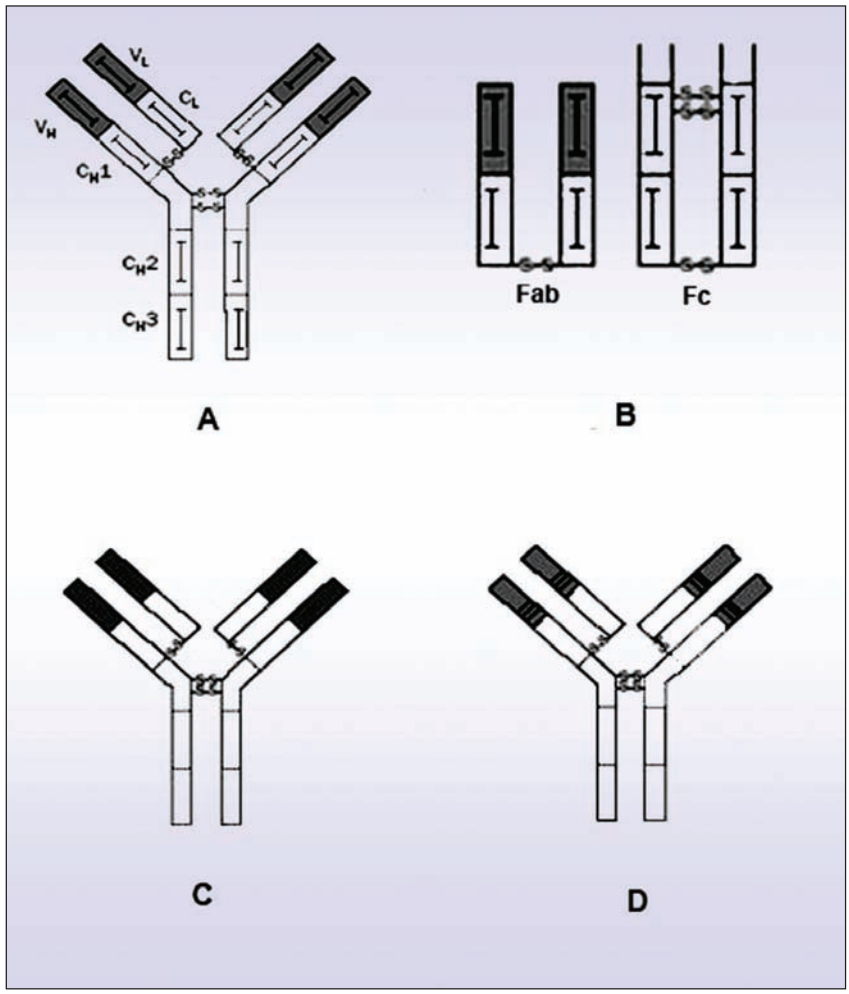

Fig. 1: Antibody structure and Function

(A) Basic antibody structure. Antibodies consist of two heavy and two light chains joined by disulphide bonds. Each chain has a variable region (VH and VL) and one or more constant regions (CH and CL).
(B) Antibody fragments. Cleavage of antibodies with the enzyme papain generates a small antigen-binding fragment (Fab) and a large crystallisable fragment (Fc). In the intact molecule, the Fc region is the biologically active portion of the antibody.
(C) Chimeric antibodies consist of murine variable regions fused to human constant regions.
(D) Humanised antibodies consist of human constant regions and variable regions, with six murine sequences (black stripes) called complementarity-determining regions or hyper variable regions.

B. Monoclonal Antibodies

The first Mabs, derived from mice, have several shortcomings. Patients treated with murine Mabs handle this construct as a foreign protein and develop a brisk human antimouse antibody (HAMA) response. HAMA will cause rapid clearance of the Mab, poor tumour penetration, as well as hypersensitivity reactions. By integrating components of human immunoglobulin into murine antibodies, new molecules with improved ability to trigger *in vivo* immune pathways in humans and be administered on a repeating schedule have been developed. These recent humanized Mab constructs have different pharmacokinetic properties compared with murine Mabs in humans.

Chimeric Mabs are 65–90% human protein and fuse the murine antibody variable region with a human IgG1 constant region, which allows for functional complement activation and ADCC in humans. Chimeric antibodies will still induce HAMA responses. Partially humanized and deimmunized Mabs, variations of chimeric Mabs, are 95% human protein and are composed of a few critical residues involved in the antigen binding site from the murine antibody, or modified murine variable domains containing non-immunogenic amino acid

sequences,respectively. To prevent any HAMA response, fully humanized Mabs containing only human protein sequences have been developed from mice that have had human immunoglobulin genes placed in their genome. To denote the different constructs of Mab, the suffixes umab (e.g. panitumumab), momab (e.g. tositumomab), ximab (e.g. cetuximab) and zumab (e.g. trastuzumab) are used. In addition, through chemical and recombinant technologies, unique molecules have been developed from antibody components. Examples include bispecific antibodies, Fab fragments, Fsc (single chain) as well as others, which have potential pharmacodynamic advantages and disadvantages over Mabs.

Therapeutic Mabs may be divided into three main classes based upon their mechanism of action[2] (Fig. 2):

(i) Mabs as directed targeted therapy: these Mabs either block or stimulate a particular cell membrane molecule (e.g. growth factor signal receptor) or ligand [vascular endothelial growth factor (VEGF)] and thereby inhibit tumour growth or activate effector cells;

(ii) Cytotoxicity by chaperoning cytotoxic molecules (immunoconjugates): these Mabs are conjugated to various cytotoxic molecules/ atoms including chemotherapy or radioisotopes such as ^{90}yttrium, which is in clinical use, cellular toxins such as diphtheria toxin or biological agents such as interferon (IFN);

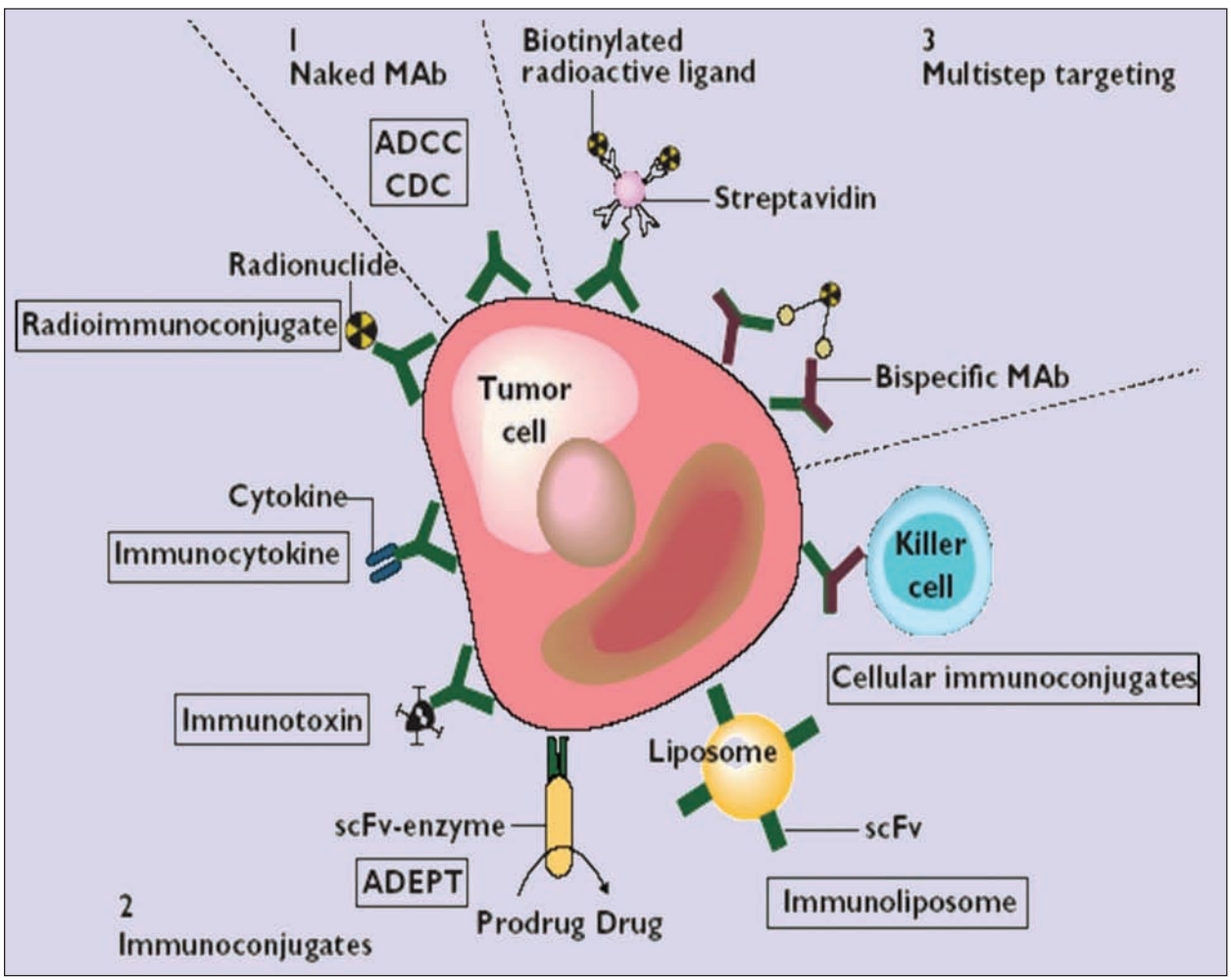

Fig. 2: Mechanism of Action of Monoclonal Antibodies [2].

(iii) Modulating an immunological mechanism: in this case, Mabs exert their cytotoxic effects by ADCC or complement-dependent cytotoxicity. These Mabs may also have some non-immunological mechanisms of action, including induction of target cell apoptosis.

C. General Clinical Pharmacology of Mabs

The IgG framework, used for the majority of therapeutic antibodies, has a molecular weight of 150 kDa and has two identical antigen binding sites.

D. Pharmacokinetics

The rapeutic antibodies can be administered via several different routes: subcutaneously, intramuscularly and intravenously. Antibody given by intramuscular (i.m) or subcutaneous (s.c) routes is primarily taken up from the interstitial fluid by the lymphatic channels via convection or passive movement with lymph fluid. Time to maximum plasma concentration ranges from 1–8 days and differs from antibody to antibody. Due to tissue degradation of antibody, between 50 and 100% of the Mab dose is available for absorption via these routes.

The intravenous (i.v.) route, in general, is preferred because of the 100% bioavailability. The i.v. route can also incur toxicity related to the rate of infusion. Once in the systemic circulation, Mabs enter the extra vascular compartment (interstitial fluid, tissue) passively, primarily through convection, and via receptor mediated pathways. Thus, the interstitial pressure of tumors can affect uptake. Interstitial pressure is typically elevated in epithelial cancers and less so in hematological malignancies. Tissue retention of Mab is influenced primarily by binding and affinity to its target, as the physical forces for tissue elimination are greater than tissue uptake. By way of varying amounts of human and mouse components, different Mab constructs be have differently when given to humans. Chimeric antibodies typically have a half-life of 4–15 days, humanized from 3 to 24 days, and recombinant human 11–24 days. HAMA response develops 7–10 days following exposure to murine antibody in humans and plays a more important role in elimination.

E. Safety Overview of Mabs

Mabs toxicity profile is related to the specificity of the target molecule and that molecule's function, as well as to the Mab construct and isotype. The majority of toxicities observed with therapeutic Mab are related to the target antigen. The most common nontargeted toxicity among nonconjugated therapeutic Mabs is a hypersensitivity reaction, which can be modified in part by the rate of infusion.

F. Specific Mabs in Clinical Use

A partial list of the FDA-approved, clinically available Mabs in use to treat human malignancies is shown in Table 1.[2]

i) *Mabs as targeted therapy*

Trastuzumab is a humanized IgG1k monoclonal antibody that binds the extra cellular domain of the HER2 (c-erbB2) receptor, a member of the epidermal growth factor receptor (EGFR) family that plays a pivotal role in growth, differentiation and cell survival. HER2 is over expressed in 25–30% of breast cancers and is associated with a poor prognosis. Blocking HER2 with trastuzumab can arrest the breast cancer cell in G1 phase of the cell cycle. Clinical trials in breast cancer patients have demonstrated the effectiveness of trastuzumab only in patients with tumors that over express HER2 protein. Over expression is typically determined by IHC or FISH.

Recent data from a randomized clinical trial (CALGB 150002) found an improved response rate in metastatic breast cancer patients with low levels of HER2 expression as defined by FISH negative, polysomy 17 positive. In patients with metastatic breast cancer, trastuzumab is given as a 4 mg kg-1 loading dose followed by 2 mg kg-1 weekly until disease progression. Objective clinical response rate to single-agent trastuzumab therapy in chemotherapy failures are a modest 15% (4% complete response). However, when used in combination with chemotherapy (anthracycline plus cyclophosphamide or paclitaxel), trastuzumab significantly improves the objective response

Table 1[2]: List of FDA-approved therapeutic monoclonal antibodies

Generic/ Trade Name	Target Antigen	Antibody Type	Approved Use	Proposed Mechanism of action
Alemtuzumab	CD52	Humanized IgG1k	B-cell chronic lymphocytic leukaemia in patients who have been treated with alkylating agents and have failed fludarabine therapy	Antibody-dependent lysis of leukaemic cells
Bevacizumab	VEGF	Humanized IgG1	Metastatic carcinoma of the colon or rectum first-line treatment of patients with unresectable locally advanced recurrent or metastatic nonsequamous nonsmall cell lung cancer metastatic breast cancer	Binds to all active forms of VEGF and inhibits angiogenesis
Cetuximab	EGFR	Chimeric IgG1	EGFR expressing metastatic innotecan-refractory colorectal carcinoma locally advanced head and neck cancer	Competitively inhibits legend binding leading apoptosis. Decreases autocrine production of growth factors
Gemtuzumab ozogamicin	CD33	Humanized IgG4k	CD33+ AML in first relapse, >60, not candidates for cytotoxic chemotherapy	Calichemicin released in lysosomes, binds DNA resulting in double strand breaks and subsequent cell death
^{90}Y-Ibritumomab tiuxetan	CD20	Murine IgG1k	Relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma, including patients with rituximab-refractory follicular non-Hodgkin's lymphoma.	Beta emissions induce cellular damage through formation of free radicals, Naked antibody induces apoptosis
Panitumumab	EGFR	Humanized IgG2a	EGFR expressing metastatic colorectal cancer	Competitively inhibits ligand binding leading apoptosis
Rituximab	CD20	Chimeric IgG2a	First treatment for follicular lymphoma Refractory indolent CD20+B-cell non-Hodgkin's lymphoma First-line treatment for diffuse large B-cell lymphoma	ADCC,CDC, induction of apoptosis
Trastuzumab	HER2 (c-erbB2) receptor	Humanized IgG1k	HER2 overexpressing metastatic breast cancer	Downregulation of HER2, inhibition of intercellular signaling, induction of apoptosis, ADCC
131-I Tositumomab	CD20	Murine IgG2aλ	CD20+ follicular, non-Hodgkin's lymphoma, with and without transformation, whose disease is refractory to nituximab and has relapsed following chemotherapy	Ionizing radiation from the 1131 apoptosis, CDC, ADCC

ADCC, antibody-mediated cellular cytotoxitiy; CDC, complement dependent cytotoxicity; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; FDA, Food and Drug Administration; AML acute myeloid leukemia.

and survival of patients with metastatic breast cancer compared with chemotherapy alone [3].The median time to progression was 7.4 months in the trastuzumab group compared with 4.6 months in the chemotherapy only group. The combination group also showed an overall response of 50% compared with 32% and a longer duration of response of 9.1 months compared with 6.1 months. There was an overall improvement in survival of 25.1 months for trastuzumab plus chemotherapy compared with 20.3 months for chemotherapy alone, corresponding to a 20% reduction in risk of death in patients treated with trastuzumab plus chemotherapy.

Subsequent studies have evaluated trastuzumab in addition to adjuvant multiagent chemotherapy in HER2+ early-stage breast cancer patients. In the herceptin adjuvant trial (HERA), trastuzumab was given every 3 weeks for 1 or 2 years after adjuvant chemotherapy and was compared with surveillance following adjuvant chemotherapy. The 2-year disease-free survival was 86% in the trastuzumab group compared with 77% in the observation group, with a corresponding hazard ratio (HR) of 0.54 ($P < 0.0001$) [4]. NSABP B-31 and NCCTG N9831 were two adjuvant studies that confirmed the benefit of trastuzumab in this setting. The 3-year disease-free survival was 87% in the trastuzumab group compared with 75% in the control ($P < 0.0001$), with a corresponding HR of 0.48.

Generally, trastuzumab is well tolerated; adverse effects include non target-related chills and/or fever, and rarely hypotension during the initial infusion.

Slowing the rate helps to ameliorate these symptoms. In randomized clinical trials, infections were seen in a slightly higher frequency in the trastuzumab group, 47% *vs.* 29% in the chemotherapy only group. Cardiac dysfunction presenting as congestive heart failure is seen more commonly when trastuzumab is administered with an anthracycline based chemotherapy regimen and less so with cyclophosphamide or paclitaxel [3]. Overall, trastuzumab is associated with a threefold increase in grade III or IV cardiac toxicity, and careful monitoring of ejection fraction using either multiple gated acquisition scan or echocardiogram is warranted.

Cetuximab is a chimeric IgG1k Mab directed against the ligand binding site of the EGFR and competitively inhibits EGF binding, leading to cell growth inhibition and apoptosis. EGFR is a transmembrane receptor tyrosine kinase expressed on normal tissue and is up regulated by transforming growth factor-alpha (TGF-a) and EGF as well as radiation. Down regulation of TGF-á, amphiregulin, angiogenic factors such as VEGF, fibroblast growth factor and interleukin-8 occurs when endogenous ligand binds to EGFR.

EGFR is over expressed in many tumour types, including 25–80% of colorectal carcinomas, where it is associated with advanced disease and poor prognosis [5]. Cetuximab is approved for use in combination with irinotecan for patients with EGFR expressing colorectal carcinoma that is refractory to irinotecan and as a single agent in patients who are intolerant to irinotecan.Cetuximab is given intravenously at 400 mgm-2 loading dose followed by 250 mgm-2 weekly until disease progression or unacceptable toxicity. Response rates in colorectal cancer to cetuximab and irinotecan in irinotecan-refractory metastatic colorectal cancer were doubled (22.9%) compared with cetuximab alone (10.8%). TTP improved to 4.1 months *vs.* 1.5 months whereas duration of response and median overall survival were not different. The best predictor of response was the development of acneiform skin rash. The level of EGFR expression did not predict for response (positive staining for expression of EGFR was defined as >1% partial or complete membranous staining of any intensity).

The justification for cetuximab use in head and neck cancer is both the over expression of EGFR and the induction of EGFR by radiation. In a randomized study of stage III and IV nonmetastatic head and neck squamous cell cancer, patients received either radiation or radiation with weekly cetuximab. Loco regional control, progression-free survival (PFS) and overall survival were significantly improved in the combination arm. The addition of cetuximab to radiation did not affect the radiation-related toxicity [6].

In general, cetuximab was well tolerated. Nontargetspecific toxicities include anaphylactic reactions, which occur in about 1% of patients. Skin toxicity is related to interaction of cetuximab with its EGFR receptor and was the most common side effect, including an acne-like rash that occurred in approximately 80% of patients. The hypomagnesaemia appears also to be related to the interaction with the target antigen, as it has been described in other EGFR Mab targeted therapy and is seen in approximately one-quarter of patients and may be severe and lead to symptoms.

Panitumumab is a fully human IgG2a Mab directed against the extra cellular domain of EGFR. It competitively inhibits EGF and TGF-á binding to EGFR and leads to internalization of the receptor. Its mechanism of action is similar to that of cetuximab, although it has a higher affinity for the receptor. Panitumumab is approved for monotherapy in colorectal cancer patients who have already failed prior chemotherapy and whose tumors show 1+ EGFR expression by immunohistochemistry in >1% of the tumour cells. Panitumumab is given intravenously at 6mg kg-1 over 1–1$^{1/2}$ h every 2 weeks. A Phase III randomized study comparing panitumumab (6 mg kg-1 every 2 weeks) with best supportive care (BSC) to BSC alone in metastatic colorectal cancer patients who had failed prior chemotherapy showed a 10% PR rate for panitumumab compared with 0% in the BSC arm [7].

To determine the survival effect of panitumumab, PFS analysis was done with and without including responding patients.

As with cetuximab, panitumumab causes skin toxicity as the most common side-effect, including conjunctivitis. For persistent grade 3 or 4 skin toxicity that improves to grade 2 or less, the dose of panitumumab can be reduced by 50% and re-escalated if the skin toxicity does not recur. Hypomagnesaemia has also been described in nearly 40% of patients and may be severe and lead to symptoms. Other common side-effects include diarrhea, cough and fatigue.

Bevacizumab is a humanized monoclonal IgG1 antibody that binds and neutralizes all biologically active forms of VEGF-A, preventing it from interacting with its receptors on the surface of endothelial cells. Bevacizumab does not neutralize VEGF-B or VEFG-C. VEGF is a proangiogenic glycoprotein produced by normal and neoplastic cells and is involved with regulation of both normal and abnormal angiogenesis and tissue proliferation. Over expression has been observed in several tumour types, including colorectal and renal cancers, and is associated with invasiveness, metastasis, recurrence and prognosis. In addition to its antiangiogenic properties, bevacizumab may potentiate both cytotoxic chemotherapy and radiation therapy by enhancing delivery through alterations of tumour vasculature and interstitial pressure.

Bevacizumab, in combination with 5-fluorouracil (5FU)-based chemotherapy, is used as first-line therapy for metastatic colorectal carcinoma [8]. In this setting, 5 mg kg-1 bevacizumab plus chemotherapy was better than 10 mg kg-1.The objective response of bevacizumab plus 5FU was 40%, with a 70% improvement in PFS (9 months) and a benefit in median overall survival of 45% (10.6 months) over chemotherapy alone. Bevacizumab in combination with FOLFOX4 (5FU, leucovorin and oxaliplatin) has also been approved for the second-line treatment of metastatic carcinoma of the colon or rectum. Median survival duration and PFS were significantly improved for the bevacizumab arm, which was associated with higher incidence of grade 3 hypertension.

At the end of 2007, the Eastern Cooperative Oncology Phase III breast cancer study of paclitaxel alone or with bevacizumab in 722 untreated metastatic breast cancer patients was published and led to the approval by the FDA of bevacizumab for breast cancer in February 2008. Response rate increased by 50% (from 21 to 37%) and PFS was doubled (11.8 *vs.* 5.9 months), although there was no overall survival difference.

Bevacizumab is also used in the treatment of patients with non small cell lung or renal cell cancers. When bevacizumab is combined with carboplatin and paclitaxel for stage IIIB and IV non small cell lung cancer, there is an improvement in objective response rate, PFS (7.4 months) and in median overall survival (17.7 months). Life threatening haemoptysis or haematemesis occurred in a small number of patients with lung cancer, and this was noted in patients with centrally located tumors, cavitary or necrotic lesions and squamous cell histology [9].

In renal cell carcinoma (RCC), a large randomized placebo controlled Phase II study has reported a 92% improvement in median PFS benefit in the 10 mg kg-1 bevacizumab group (4.8 months).The results of Phase III studies with bevacizumab and IFN-alfa have confirmed the activity of the combination over IFN-alfa alone in metastatic RCC patients [10]. Bevacizumab is well tolerated, with adverse effects related to its effect on the target molecule for the most part, including bleeding, clotting, GI perforation, hypertension and proteinuria. Bevacizumab-associated GI perforation typically presents with abdominal pain, nausea and vomiting, and has been reported in approximately 1.5% of patients. Bevacizumab associated GI perforation is associated with tumors still in place in the GI tract and is not associated with history of peptic ulcer disease or diverticulitis. GI perforation is an absolute contraindication for continuing bevacizumab therapy. More recently, the syndrome of reversible posterior leukoencephalopathy (headache, seizure, feeling tired, confusion, vision problems, and elevated blood pressure) has been seen in 0.1% of patients receiving bevacizumab.

ii) *Immunological mediated cytotoxicity agents*

Rituximab is a chimeric anti-CD20 IgG1k Mab that targets the CD20 antigen expressed on the surface of >90% of malignant and normal B lymphocytes. CD20 is expressed at lower density on CLL cells. The CD20 antigen is an attractive target, in that it is not expressed on stem cells, does not circulate in the plasma, is not shed from the cell surface after binding and is not internalized or down regulated. The proposed mechanisms include ADCC; complement mediated cytotoxicity, as well as growth inhibition, cell cycle alteration and apoptosis via direct binding to CD20. In addition, *in vitro* data suggest that it may sensitize lymphomas to the action of cyclophosphamide, doxorubicin, and vincristine and prednisone chemotherapy. Approximately 50% of patients with low-grade or follicular CD20+ lymphoma will have an objective response (6% complete response rate) [11].The median time to progression for responders and duration of response were approximately 13 and 12 months, respectively. Rituximab has been studied in several other hematological disorders. In diffuse large B-cell lymphoma, rituximab combined with chemotherapy significantly improved the complete response rate (CR = 76%), as well as event-free and overall survival. In addition, rituximab and chemotherapy improved the CR rate by 3.9-fold (CR = 34%) and the objective response rate by 25% (OR = 94%) in mantle cell lymphoma, although no overall survival advantage was observed [12].

Additional studies have suggested a role of rituximab in the treatment of chronic lymphocytic leukemia as well as in refractory idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura and autoimmune hemolytic anemia. Maintenance rituximab has been studied in several randomized trials, both when used as a single agent and in combination with chemotherapy. In a recent study of advanced-stage relapsed or refractory follicular or mantle cell lymphoma, patients received rituximab plus chemotherapy and were then randomized to receive maintenance rituximab 375 mgm-2 weekly for 4 weeks at 3 and 9 months after achieving a PR or CR .The median PFS was 17 months for the observation arm, whereas the median PFS for the maintenance arm has not been reached at 3 years of follow-up. Overall survival was improved with a trend toward significance at 3 years of 77% in maintenance arm *vs.* 57% in observation [13]. The optimal schedule of maintenance rituximab and which subsets of non-Hodgkin's lymphoma (NHL) patients benefit most from this treatment remain to be clearly defined. Rituximab is well tolerated and can be administered to most patients regardless of age or performance status.

Adverse events thought to be mediated by the effector function of the Mab are infusion-related and commonly include fever, chills, flushing, and, less commonly, bronchospasm and hypotension. They typically occur during the first hour of infusion and last only 1–2 h. Anemia, thrombocytopenia and neutropenia are seen in approximately 10% of patients and are mostly grade 1–2 in severity. The US FDA issued an alert regarding the development of progressive multifocal leukoencephalopathy, a rare demyelinating disease caused by human polyomavirus (JC) of oligodendrocytes, which caused death in two patients on rituximab.

Alemtuzumab is a recombinant humanized IgG1k Mab against CD52. Human CD52 is a glycosylphosphatidylinositol- anchored antigen expressed on normal and malignant B and T lymphocytes as well as on some male genital tract epithelial cells. CD52 is not expressed on haematopoietic stem cells. Alemtuzumab is used for the treatment of B-cell chronic lymphocytic leukemia (B-cell CLL) after treatment failure with alkylating agents and fludarabine. The agent is particularly effective in 17p deletion patients. Alemtuzumab was titrated up to 30 mgm-2 three times a week for up to 12 weeks. The overall response was 33%, with 2% achieving complete remission. The median time to response was 1.5 months, median time to progression was 4.7 months overall, 9.5 months for responders. Median survival was 16 months overall and 32 months for responders.

Alemtuzumab is being explored as first-line therapy in patients with B-cell CLL. A preliminary study has shown a high overall response rate of 87%; 19% complete response was observed. Phase III study has compared single agent alemtuzumab with chlorambucil for first-line therapy in B-cell CLL with markedly superior response rates and survival [14].

The common adverse events to alemtuzumab are thought to be mediated by the effector function during infusion and include rigors, fever, nausea, vomiting, rash, pruritus, urticaria, dyspnoea, diarrhea and hypotension. Premedication with antihistamines, paracetamol, antiemetics, meperidine and corticosteroids is used to reduce infusion-related toxicity. Starting at a lower (10 mg) dose with escalation on subsequent days has also been useful in reducing infusion-related toxicity. Patients are at risk for opportunistic infections both during treatment and 2 months post treatment, with reactivation of cytomegalovirus being most frequent. All patients should receive antibiotic and antiviral prophylaxis.

iii) *Conjugated monoclonal antibodies*

Rituximab therapy has been successful, but tumour cell resistance still plays a significant role in treatment failure. Mechanisms of resistance include inadequate serum/tissue antibody concentrations, limited access to tumour cells with bulky disease, defective tumour effector mechanisms, and genetic polymorphisms in the FcγRIII gene, which may lower the binding affinity of the antibody for the Fc receptor.

One approach to overcome tumour resistance has been the development of conjugated antibodies. Given lymphoma's sensitivity to radiation, attaching a radionuclide to a tumour-specific antibody is a rational combination targeting approach. This technology is termed radioimmunotherapy (RIT). The β emissions from radionuclide that are commonly used in RIT have a cytotoxic effect of approximately 100–200 cell diameters and therefore are lethal to neighbouring tumour cells that may be inaccessible to antibody or may not express the target antigen. This is termed as the 'cross-fire' or 'by-stander' effect.

^{90}Y-ibritumomab tiuxetan is an immunoconjugate composed of ibritumomab, a murine IgG1k anti-CD20 Mab covalently bonded to the linker-chelator tiuxetan, which provides a high-affinity chelation site for the ^{111} indium used for imaging or ^{90}yttrium used for therapy. The Mab was left in its murine form in order to accelerate clearance and limit the effects of prolonged irradiation exposure. In follicular B-cell NHL refractory to rituximab treatment, 90Y-ibritumomab tiuxetan therapy demonstrated an overall response rate of 74%, with 15% of patients achieving a complete response. Median time to progression was 6.8 months and the median duration of response was 6.4 months. 90Y-ibritumomab tiuxetan appears to provide an improved response rate to rituximab in patients with relapsed or refractory low-grade lymphoma, follicular lymphoma or transformed B-cell NHL, heavily pretreated low-grade follicular NHL with or without transformation [ORR was 80% (30% CR) in the ^{90}Yibritumomab tiuxetan arm] compared with 56% (16% CR) for the rituximab-alone arm [15].

There was substantially greater hematological toxicity for ^{90}Yibritumomab tiuxetan-treated patients in both studies. The dose (0.4 mCi kg-1 actual body weight) of ^{90}Yibritumomab tiuxetan is given only after the biodistribution of ^{111} In-ibritumomab tiuxetan is determined by whole-body γ-camera images to be adequate. The $t^{1/2}$ of 90Y-ibritumomab tiuxetan is 30 h. Over 7 days, a median of 7.2% of the injected activity was excreted in the urine.

Tositumomab and ^{131} I-tositumomab:

^{131} I-tositumomab is comprised of tositumomab, a murine IgG2al murine Mab directed against CD20 covalently linked to ^{131} iodine. Tositumomab is used for therapy of CD20+ follicular NHL, with and without transformation that is refractory to rituximab and has relapsed following chemotherapy. Response rates to ^{131} I-tositumomab in low-grade or transformed low grade chemotherapy-refractory CD20+ B-cell lymphoma were 65% and were better than additional chemotherapy. Tositumomab has a 65% overall response rate in low grade or transformed lymphoma failing rituximab therapy, with a median duration of response of 14.7 months. A complete response was

seen in 38% of patients. The common toxicities relate to severe bone suppression [16]. More than 70% of patients experience National Cancer Institute (NCI) Common Toxicity Criteria grade 3–4 cytopenias. Less than 10% of patients experience hypersensitivity, including life-threatening anaphylactoid reactions. Infusion-related pyrexia, rigors, hypotension dyspnoea and bronchospasm are seen in the first 48 h and the fever and rigours can be seen after as long as 14 days. Hypothyroidism related to the ^{131} I can develop, and thyroid blocking agents are suggested.

The median clearance following administration of 485 mg of ^{131}I-tositumomab in 110 patients with NHL was 68.2 mg h-1 (range 30.2–260.8 mg h-1).Those patients with high tumour burden, splenomegaly or bone marrow involvement are noted to have a faster clearance, shorter half-life and larger volume of distribution. The overwhelming majority of the elimination of iodine 131 I occurs through renal excretion. 131 I decay accounts for a minority of elimination. 131 I has a half-life of 8 days. Five days following the dosimetric dose, the whole body clearance was 67% of the injected dose.

Gemtuzumab ozogamicin is a humanized IgG4k anti- CD33 Mab covalently linked to a semi synthetic derivative of the potent cytotoxic antibiotic calicheamicin. Gemtuzumab ozogamicin binds to the CD33 receptor, resulting in complex formation and internalization. Once internalized, the calicheamicin derivative is thought to be released in the lysosomes and then binds to DNA, resulting in double strand breaks and subsequent cell death. In addition, calicheamicin may cause cell death through a caspase mediated pathway. CD33 is expressed on approximately 90% of acute myeloid leukemia myeloblasts as well as normal myeloid precursor cells. CD33 expression is down regulated with maturation of myeloid cells, which results in a low level of expression on circulating granulocytes and tissue macrophages. Gemtuzumab ozogamicin is used in the treatment of CD33+ acute myelogenous leukemia (AML) in first relapse, and those >60 years old and not considered candidates for cytotoxic chemotherapy. Gemtuzumab ozogamicin is given intravenously at a dose of 9mgm-2 over 2 h for up to three doses, with a minimum of 14 days and a maximum of 28 days between doses. Complete response as defined by US NCI consensus criteria was seen in 16% of patients.CR plus CR with incomplete platelet recovery were reported in 30% of the patients [17].

Common transient and reversible adverse events related to the infusion include chills, fever and hypotension. These events occurred despite use of prophylactic paracetamol and antihistamines. Treatment-related adverse events include grade 3–4 neutropenia, thrombocytopenia, neutropenic fever, infection and sepsis, as well as hemorrhage including intracranial bleed and hemorrhagic death in a small percentage. Additional serious grade 3–4 toxicity includes hyperbilirubinemia; anemia and transaminitis.Gemtuzumab ozogamicin has been associated with increased risk for veno-occlusive disease in patients who went onto stem cell transplantation.

G. Future Directions

1. Using Mab cocktails may provide improvement in clinical response in cancer patients. One such attempt has been studied in a randomized Phase II design of cetuximab and bevacizumab alone or with irinotecan in irinotecan-refractory colorectal patients, the BOND-2 Study.
2. Use Mabs in conjunction with small molecule TKI agents to provide either vertical (within the same signal pathway) or horizontal (across different signal pathways) blockade. One such approach in RCC employed bevacizumab to block the VEGF pathway and erlotinib, a signal transduction inhibitor of the EGFR.
3. Mab constructs (ipilimumab and ticilimumab) have been used to block T-lymphocyte receptor (CTLA4) responsible for negative regulation of cellular immune responses.
4. Newer monoclonal antibodies are in various phases of development. (Table 2 [18]).

H. Conclusions

Targeted therapies represent a paradigm shift in the

Table 2: New monoclonal antibodies in various phases of development

Antibody	Target	Trial	n	Setting
Alemtuzumab(subcutaneous)	CD52	Phase I	41	Untreated CLL
		Phase III	297	Untreated CLL (vs chlorambocil)
Epratuzamab	CD22	Phase I/II	56	Refractory recurrent aggressive lymphoma
		Phase I/II	55	Refractory recurrent indolent lymphoma
CMC 544	CD22	Phase I	34	Relapsed/refractory lymphoma
Galiximab	CD80	Phase II	37	Relapsed/refractory FL
Lumiliximab	CD23	Phase I	46	Refractory/recurrent CLL
Ofatumumab	CD20	Phase I/II	33	Relapsed/refractory CLL
		Phase I/II	40	Relapsed/refractory FL
Zanolimumab	CD4	Phase II	47	Refractory $CD4^+$ CTCL
		Phase II	15	Refractory $CD4^+$ PTCL
SGN 30	CD30	Phase II	20	Refractory/recurrent systemic ALCL
		Phase II	17	Refractory/recurrent cutaneous ALCL
		Phase II	15	Refractory/recurrent HD
MDX 060	CD30	Phase I/II	48	Refractory/recurrent $CD30^+$ lymphoma
HuM195	CD33	Phase II	50	Refractory/recurrent AML

treatment of cancers. Unlike traditional chemotherapy drugs they seek to target qualitative or quantitative abnormalities specific to cancer cells, often involving receptor mediated signaling pathways . They represent the fruits of the enhanced understanding of the underlying mechanisms of the development and progression of the transformed cells. This understanding has been possible due to conceptual and technological advances in the fields of molecular biology, genomics, proteomics, rational drug design, protein engineering and others.

This review discussed the present status of therapeutic monoclonal antibodies that represent one of the most important classes of targeted therapies. These drugs have significantly contributed to the improved outcome in solid tumors like colorectal, breast, lung and head and neck carcinomas and hematological malignancies like B cell non-Hodgkin lymphomas. However these drugs still need to be combined with chemotherapy drugs in most instances. Moreover, although they have improved the survival in advanced metastatic cancers, they are still far from being curative in these situations. The high cost of some of these drugs necessitates a continuing appraisal of their role in well conducted controlled clinical trials.

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"Nil Per Oral after Midnight": Is it Necessary for Clear Fluids?

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Introduction

Long fasting hours prior to surgery pose great discomfort to the patient. Despite recent guidelines stating that it is appropriate to reduce the interval of clear fluid ingestion to 2 hours prior to surgery[1], it is common practice to follow nil per oral, from midnight, for both solids as well as clear fluids. Decreasing the fasting period enhances the quality and efficiency of anaesthesia care by decreasing the cost, increasing patient satisfaction and avoiding delays and cancellations. There is also a decrease in the risk of dehydration and hypoglycemia and thereby decrease in the perioperative morbidity.

Previous studies have shown that pH less than 2 and volume of gastric aspirate more than 25ml (0.4ml/kg) predispose a patient to pulmonary aspiration[2], hence a strict overnight fasting regimen was instituted. Recent studies have shown, that prolonged withholding of oral fluids does *not* improve gastric pH or volume, and may even worsen it.[3] In an attempt to reduce the fasting \hours of a patient preoperatively without increasing the risk of pulmonary aspiration, it was decided to assess the safety of ingestion of 150ml of water 2 hours prior to surgery in patients undergoing general anaesthesia with endotracheal intubation.

Materials and methods

After written informed consent, 100 ASA I and II patients between 12-60 years, posted for elective orthoaedic, gynaecologic, otolaryngologic and geneal surgery were divided into 2 groups. Emergency surgeries, patients with history of acid peptic disease, diabetes mellitus, obesity, pregnancy, hiatus hernia[4], as well as those routinely taking any medications that affect gastric motility or secretion, were excluded from the study. Sex, age, weight, type of surgery, duration of fasting and interval between ingestion of water and surgery was documented. Group I was kept fasting overnight, Group II was given 150ml water 2 hours prior to surgery. Patients were premedicated with midazolam and pentazocine, general anaesthesia was induced using intravenous pentothal followed by a muscle relaxant vecuronium. A 18G and 16G Ryle's tube was inserted in male and female patients respectively after intubation and its position was confirmed by auscultation over the epigastrium for insufflated air. Gastric aspirate was obtained through a 20ml syringe with the patient supine with an assistant massaging the upper abdomen, as well as with changes in position like Trendelenburg, left lateral and right lateral positions to facilitate maximal aspiration. Volume of aspirate was noted and pH measured using a standardized pH strip. Results have been given as mean $\pm$ *SD* and ranges where appropriate. Data has been analysed using Student's t-test. Differences were considered statistically significant, if p values were <0.05.

Table 1: Patient Characteristics

	Group I	Group II
Age (years):	42+12.96	44+16.42
Sex: M/F	9 / 21	14 / 16
Weight (kg):	51+9.21	53+7.84
Ingestion- Surgery Interval (Mins)	742+70.08	130+6.64

Except for sex, values are expressed as Mean ± SD

Table 2: Volume and pH

	Group I	Group II
pH	1.7+0.28	1.6+0.26
extremes	1.5, 2.5	1.5, 2.5
Gastric Fluid Volume(ml)	17.1+8.21	5.5+3.70
extremes (ml)	5, 42	2, 18

Table 3: Incidence of Risk Factors*

	Group I	Group II
Volume > 25ml	4	0
pH < 2.5	29	29
Volume >25ml+pH <2.54		0

**Residual gastric fluid volume > 25ml and pH < 2.5*

Results

There were no significant differences between the groups in terms of weight, age, sex (Table 1). The volume and pH of gastric content has been shown in Table 2. Patients who were kept fasting overnight (Group I) had an average fasting time of 12 hours. The ingestion- surgery interval for Group II was averaged at 2 hours. Patients who had 150ml of water (Group II) had lesser volume of gastric aspirate than that of Group I, which was statistically significant, whereas the pH was found to be in the same range for both the groups. The patients with the high risk factors of gastric fluid volume >25ml and pH < 2.5 is shown in table 3. In Group I, 4 patients were found to have a combination of both factors, while none were seen in Group II.

Discussion

Pulmonary aspiration of gastric contents during anaesthesia is a *rare* event occurring in approximately 1 in every 3000 general anaesthethetics, yet is a significant cause of anaesthesia related deaths[5]. The severity of pulmonary damage is related to both the volume and pH of the gastric fluid, a combination of volume of > 25ml with pH < 2.5 being considered lethal[2]. Hence any safety measure that *reduces* this hazard was preferred, so the routine preoperative practice of "nothing by mouth after midnight" has been followed. But the "nil per oral" order is blindly applied to both liquids and solids and has become engrained in our anaesthetic practice. The time required for solid food to liquefy and enter the small intestine depends on the type of food ingested, (being shorter for carbohydrates and proteins than for fats and cellulose) and the food particle size.[6] Complete emptying of solids from the stomach takes 3-6 hours, but may be prolonged by fear, pain or opioids[6]. So it is appropriate that no solid food be eaten on the day of surgery. However the gastro-oesophageal emptying of liquids wherein more than 90% of even a 750ml bolus of isotonic saline empties within 30 minutes in most patients.[7]

At the time of induction of anaesthesia, gastric fluid volume is quite variable in normal people. Even if the patient has fasted, the stomach is not totally empty. On an average, 35 ml of gastric fluid volume remains in the stomach and can even be as much as 200ml. [8] Comparing this to the traditional cut-off of gastric fluid volume >25 ml and pH < 2.5, 30-60% patients would be at risk of pulmonary aspiration[9], but the incidence is as low as 1 in 3000. Passive regurgitation of gastric contents can occur, only if intragastric pressure exceeds the protective tone of the lower oesophageal sphincter. For pulmonary aspiration to occur, the protective airway reflexes must be abolished[10].

Our study was undertaken to determine whether a 2 hour fast with clear fluids was safe for patients.

Clear fluids would include black tea, coffee, fruit juices without any particulate matter and water [9]. We chose 150ml of water to be given prior to surgery. We used a Ryle's tube for aspiration of gastric contents which is a well accepted method for assessment[11]. Our study confirmed the results of previous studies that even after 11-13 hours of fasting, a large number of patients had gastric pH < 2.5 and gastric fluid volume >25 ml[12, 13].

Patients who received 150 ml water actually had decreased gastric fluid volume which was statistically significant, as seen in another study[3], whereas the pH remained unaffected. Studies have also shown, that giving clear fluids increased patient comfort and decreased anxiety and thirst.[3]

We conclude that it is safe to conduct general anaesthesia in patients who have ingested 150 ml of water 2 hours prior to surgery. Prolonged withholding of oral fluid does not improve gastric fluid volume and pH. Clinicians should appraise this evidence and adopt the recent American Society of Anesthesiologists' guidelines, which recommend an evolution from the indiscriminate "Nil per oral after midnight" blanket fasting policy. However, the customary 8 hour fasting should be followed for patients at a higher risk of aspiration like in diabetes mellitus, pregnancy, obesity as more research is necessary to determine the safety in these patients. The risk of unexpected regurgitation cannot be avoided by overnight fasting too, so anaesthetists must always be prepared to deal with these complications.

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Amino Acid Supplementation to Mothers to Improve Birth Weight

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Introduction

Intrauterine growth restriction (IUGR) refers to a condition in which a foetus is unable to achieve its genetically determined potential size. Thus, it excludes the foetuses that are constitutionally small for gestational age.

It is estimated that 3 to 10 percent of newborns are growth restricted.

IUGR occurs when gas exchange and nutrient delivery to the foetus are not sufficient to allow it to thrive *in utero*. This results in alteration of normal foetal physiology and a spectrum of complications. These complications include foetal morbidity and mortality, need for induction of labour, foetal compromise during labour and increased incidence of caesarean delivery.

Neonates who survive the compromised intrauterine environment are at increased risk of hypoglycaemia, hypocalcaemia, hyperbilirubinemia, inadequate temperature control, necrotizing enterocolitis, meconium aspiration syndrome, respiratory distress syndrome, intraventricular bleeding, thrombocytopenia, neonatal encephalopathy and renal failure. Gardosi et al noted in 1998, that nearly 40% of stillborn foetuses that were not malformed were IUGRs.

Maternal causes of IUGR

- Protein-calorie malnutrition, smoking, substance abuse
- Chronic hypertension, Gestational hypertension
- Cyanotic heart disease
- Class F or higher diabetes
- Haemoglobinopathies
- Autoimmune disease, thrombophilias
- Uterine malformations.

Placental or umbilical cord causes of IUGR

- Placental abnormalities, Placenta previa
- Chronic abruption
- Abnormal cord insertion
- Cord anomalies
- Multiple gestations, Twin-to-twin transfusion syndrome.

Criteria for diagnosis of IUGR

Effective Foetal Weight at or below the 10th percentile on ultrasonography is used, to identify foetuses at risk of IUGR. This may include constitutionally small foetuses also. In these cases, short maternal or paternal height, the foetus's ability to maintain growth along a standardized curve and a lack of other signs of uteroplacental insufficiency such as oligohydramnios, abnormal doppler findings are considered reassuring, by the clinician.

If mother's dates are uncertain or unknown, obtaining a second growth assessment over a 2 to 4 week interval is valuable.

Screening the foetus for growth restriction

Screening for IUGR in the general population relies on symphysis–fundal height (SFH) measurements which is a routine part of antenatal care from 24 weeks until term. Two large retrospective studies suggest that decreased SFH measurements correctly diagnosed 25 to 50% of growth restricted foetuses clinically.

Single biometric measurement cannot exclude the diagnosis of growth restriction. Women have to undergo serial sonographies for confirmation of diagnosis.

Amino acids in human nutrition

Amino acids are critical to life and have many functions in metabolism. These are the building blocks of proteins. There are two types of amino acids. Non-essential amino acids are synthesized in the body while essential amino acids being not synthesized, must be supplied exogenously. Optimum ratio of amino acids is needed for synthesis of tissue proteins, enzymes

and hormones. Thus, amino acids play an important role in human nutrition.

Amino acids and IUGR

Amino acids have multiple functions in foetoplacental development. The supply of amino acids to the foetus involves active transport across the placenta and metabolism within the trophoblast. The capacity of the placenta to supply amino acids to the foetus, depends upon factors such as surface area and specific time-dependent transport system expression. After transport across the trophoblast in normal conditions, amino acids are actively incorporated into tissue proteins or oxidized.

In intrauterine growth restriction (IUGR), placental surface area and amino acid uptakes are decreased. Potential changes may occur in the insulin/IGF-I signalling pathway that includes decreased production and/or activation of specific signalling transduction protein, mammalian target of rapamycin (mTOR). This leads to a reduced protein synthesis in foetal tissues. Thus, combination of decreased foetoplacental amino acid uptake and disrupted insulin/IGF-I signalling in liver and muscle account for decreased foetal growth in IUGR.

Along with amino acids, foetus requires several other substrates for normal growth. The most important are oxygen and Glucose. Oxygen crosses placenta by simple diffusion and is necessary for formation of chemical energy in the form of adenosine triphosphate (ATP)

Glucose crosses the placenta by facilitated diffusion, utilized in the production of energy and in the provision of carbon building blocks for the synthesis of lipids, glycogen, nucleotides and other molecules.

Materials and Methods

650 antenatal women, who underwent interval growth ultrasonography at 32 – 33 weeks at BARC Hospital, were included for data analysis. Effective foetal weights were calculated. Data obtained was plotted on Thompson's graph. If the foetal weights were below 10th percentile for that gestation, (after excluding women with history of mistaken dates and constitutionally small foetuses), patients were admitted in view of IUGR. These mothers were given essential amino acid infusion therapy as per regimen mentioned.

After delivery, birth weights of newborns were noted and plotted on Thompson's graph. The birth weight percentiles were noted. Maternal, foetal and neonatal complications if any were recorded.

Therapeutic Regimen

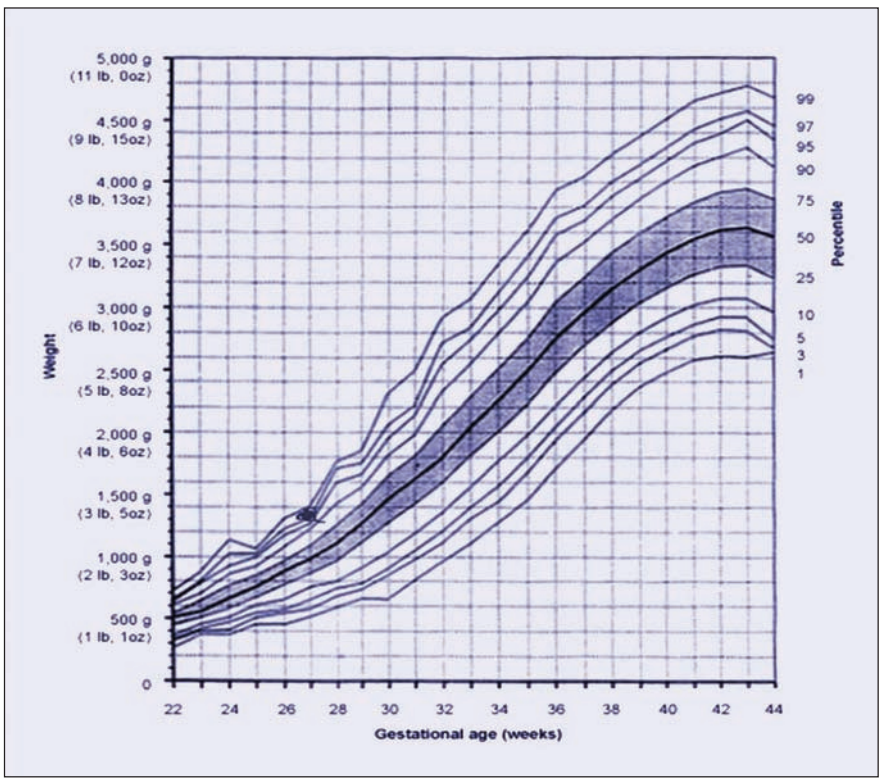
Bed rest, High protein diet

Day 1- Essential Amino acid infusion drip of 200ml over a period of one hour.

Day 2- 1L of 5% dextrose infusion with humidified oxygen at a rate of 4lit/min for 4hrs.

This therapy continued alternately till 5 doses of amino acid infusions for a total period of 10 days were completed.

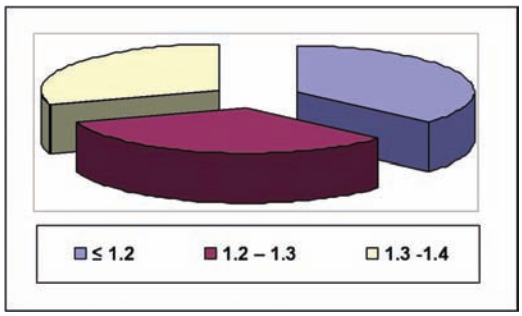
Graph (Thompson) including foetal weights



Observations

1. EFW Percentage at 32 weeks USG

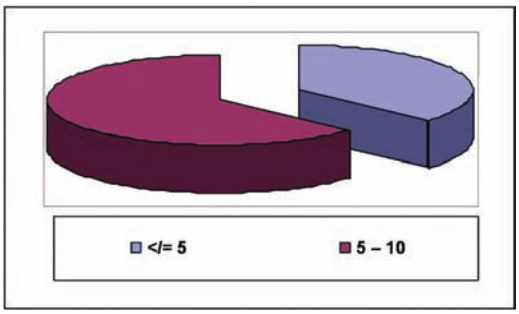
EFW (Kg)	No. of Cases	Percentage (%)
≤ 1.2	11	36.66
1.2	10	33.33
1.3-1.4	9	30
Total	30	100



- Out of 650 patients delivered over 12 months at BARC hospital, 30 patients (4.62%) had effective foetal weight (EBW) below 10th percentile at 32-33 weeks by ultrasonography.
- 9 cases (30%) had EFW between 1.3-1.4 kg,10 cases (33.33%) had EFW between 1.2-1.3kg & 11 cases (36.66%) had EFW d" 1.2 kg on ultrasonography done at 32-33 weeks.

2. EFW percentile at 32 weeks USG

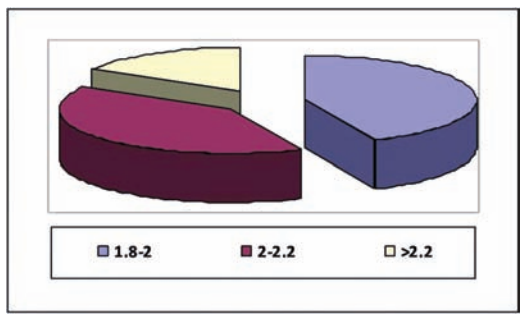
EFW Percentile	No. of Cases	Percentage (%)
</= 5	11	36.66
5 – 10	19	63.33
Total	30	100



- 19 cases (63.33%) were between 5th -10th percentile while 11cases (36.66%) were below 5th percentile.

3. Birth Weight percentage after treatment

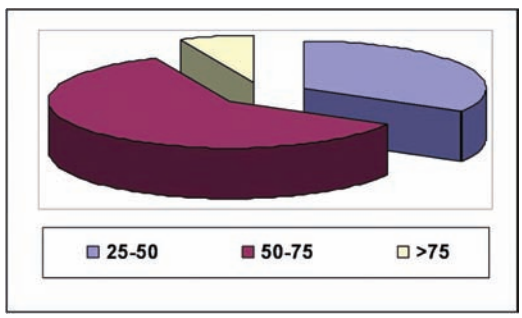
Birth Weight(Kg)	No.of Cases	Percentage (%)
1.8 – 2	13	43.33
2 – 2.2	12	40
> 2.2	5	16.66
Total	30	100



- At birth 13(43.33%), 12(40%) and 5(16.66%) had birth weight between 1.8 – 2kg, 2- 2.2kg and >2.2kg respectively.

4. Birth Weight Percentile after treatment

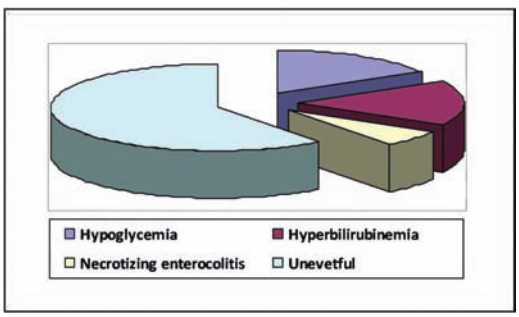
Birth Weight percentile	No. of Cases	Percentage (%)
25-50	10	33.33
50-75	18	60
> 75	2	6.66
Total	30	100



- At birth 10 (33.33%) cases, 18(60%) cases, 2(6.66%) cases corresponded with birth weight percentiles of 25-50, 50-75 and more than 75 respectively.

5. Neonatal complications

Complications	No. of Cases	Percentage (%)
Hypoglycemia	5	16.6
Hyperbilirubinemia	5	16.6
Necrotizing Enterocolitis	2	6.6
Uneventful	18	60.2



- After receiving therapy, all the neonates were born between 25th & 75Th percentile of expected birth weight.
- No neonate was born below 10th percentile of expected weight.
- No foetal mortality was noted.
- 5 neonates each had hypoglycemia & hyperbilirubinemia respectively.
- 2 neonates each had necrotizing enterocolitis.
- 60.2% newborns had no complications.

Discussion

Battaglia and Lubchenco classified *small-for-gestational-age* (*SGA*) infants as those whose weights were below the 10th percentile for their gestational age. Such infants were shown to be at increased risk for neonatal death. The neonatal mortality rate of SGA infants born at 38 weeks was 1 percent compared with 0.2 percent in those with appropriate birth-weights.

Nicolaides et al performed cordocentesis on 28 small-for-gestational-age (SGA) and 62 appropriate-for-gestational-age (AGA) foetuses. The amino acid concentrations of foetal and maternal blood were compared. Amino acid concentrations were higher in AGA foetal blood than in the maternal blood. The amino acid concentrations of SGA foetuses were lower than the concentrations found in maternal blood. The low amino acid concentration was related to decreased oxygen concentration in the foetal blood.

Economides and associates hypothesized that hypoglycaemic, growth-restricted foetuses mobilize adipose tissue and that hypertriglyceridemia is the result of lipolysis of their fat stores. Thus, amino acid deficiency is well documented as an etiology of IUGR.

Hence, high protein diet is recommended in management of intrauterine growth restriction. These proteins can be supplied orally or in cases of reduced absorption, impaired digestion or high metabolic demands as in case of late trimester of pregnancy, parental protein therapy is instituted.

Solution containing eight essential amino acids along with Glycine as a source of nitrogen is infused intravenously. This provides positive nitrogen balance in the face of growth requirements. It is recommended to administer parenteral amino acid along with 5 % glucose solution as a source of energy. Metabolic utilization of glucose spares amino acids for their anabolic action in the body and protein synthesis.

Conclusion

Identifying risk factors, screenings by regular antenatal check up and early diagnosis by serial ultrasonographies remain the cornerstones in identification of IUGR.

Maternal supplementation of amino acids has shown improvement in birth weight of suspected IUGRs minimizing the neonatal morbidity and mortality with no adverse effects.

Additional Reading

- William's Obstetrics 23rd Edition.
- Foetal Growth Restriction, emedicine Obstetrics and Gynaecology.
- Dewhurst's Textbook of Obstetrics & Gynaecology 7th Edition.
- Foetoplacental Transport and Utilization of Amino Acids in IUGR-A Review:
- *Placenta* (2005), Vol.26, Suppl. A.*Trophoblast Research* Vol.19.

Study of Prevention and Reversal of early Atherosclerotic Changes in Syndrome X Individuals

Dr. K.R. Bantwal, Dr. S. Anand, Dr. A.V. Kulkarni,
Dr. S. Kantharia and Dr. A. Chaubey

Abstract

Total number of 75 cases of syndrome x were chosen, to study the prevention and reversal of early atherosclerotic changes by applying simple lifestyle modification and appropriate medication (hypoglycaemic agents, antihypertensives) when required. Antiplatelet agents and statins were added to those who showed endothelial dysfunction.

Introduction

Atherosclerosis is a major cause of disability and death in those with insulin resistance and hyperinsulinaemia with metabolic risk factors, that are involved in the aetiology of atherosclerotic disease. It substantially increases the risk of developing coronary, cerebral and peripheral arterial vascular disease. The name derived from Greek, refers to the thickening of the arterial intima (sclerosis, "hardening") and accumulation of lipid (athere,"gruel") that characterize the typical lesion.

Atherosclerosis of the coronary arteries commonly causes angina pectoris and myocardial infarction. Atherosclerosis of the arteries supplying the central nervous system frequently provokes transient cerebral ischemia and stroke. In the peripheral circulation, atherosclerosis can cause intermittent claudication and gangrene and can jeopardize limb viability. Atherosclerosis can affect the kidney directly e.g., causing renal artery stenosis. Involvement of the splanchnic circulation can cause mesenteric ischemia and bowel infarction. Other arteries such as the internal mammary arteries seldom harbour atherosclerotic lesions.

Atherosclerosis can also cause ectasia and development of aneurismal disease with an increase in lumen calibre. This expression of atherosclerosis frequently occurs in the aorta, creating a predisposition to rupture or dissection, rather than to stenosis or occlusion.

The identification and treatment of vulnerable asymptomatic (subclinical) atherosclerotic patients are the focus of this study.

Aims and Objectives

1. Prevention and early recognition of atherosclerotic condition in syndrome x individuals.
2. To apply lifestyle changes and appropriate medication in these individuals.
3. To check for reversibility of early atherosclerotic changes with the use of statins and antiplatelet agents at the end of 2 .5 yrs of follow up.

Material and Methods

Duration of this prospective study was from July 2006 to December 2008. Total number of 75 cases of syndrome x belonging to the age group of 30 to 60 years were chosen for this study. There were no known cases of coronary, cerebral or peripheral vascular disease included in this study.

Criteria suggested by National Cholesterol Education Program (NCEP) Adult Treatment Panel III (2001) was used, to diagnose syndrome X individuals. An individual who satisfied at least 3 of the following 5 criteria was said to be suffering from syndrome X.

CRITERIA	MALES	FEMALES
1. Waist circumference	>90cm	>80cm
2. S.Triglyceride	>150mg%	>150mg%
3. HDL cholesterol	<40mg%	<50mg%
4. Blood pressure	>130/85mmHg	>130/ 85mmHg5.
5. Fasting Plasma Glucose	>100mg%	>100mg%

From each individual, relevant demographic data and lifestyle information was collected using a questionnaire. Demographic information included age, gender, marital status etc. Lifestyle information included occupation, food habits, tobacco, alcohol usage and sedentary nature of lifestyle. Anthropometric data was collected from each individual using standard methodology. Anthropometric measurements included height, weight, waist circumference (WCIR) and blood pressure. A 12 hour fasting blood biochemistry analysis was performed by the Pathology Department of BARCH. The levels of the Fasting Plasma Glucose, Total Cholesterol, High Density Lipoprotein Cholesterol, Low Density Lipoprotein Cholesterol and Triglycerides were determined.

Diagnosed syndrome X individuals were subjected to Carotid Artery Doppler performed by Radiology department BARCH to detect atherosclerotic changes. Non invasive carotid artery intima-media thickness and plaque by ultrasonography is capable of providing direct evidence of the presence and extent of atherosclerosis, prognostic information of proven value regarding the future risk of heart attack and stroke. This screening result along with risk factor assessment is used, to identify vulnerable patients and initiate therapy.

Table 1: Observation, Results & Discussion.

Total 75 syndrome x individuals belonging to various age groups by gender.

Age group (Years)	Males n=38	Females n=37
30-34	2	0
35-39	6	5
40-44	8	8
45-49	9	8
50-54	6	8
55-60	7	8

Table 2: Number (percentage) of adults suffering from syndrome x above the thresholds of the variables of syndrome x by genter

Variables	Males n=38	Females n=37
1) Waist circumference M=>90cm F=>80cm	31(81.578%)	37(100%)
2) S triglycerides =>150mg%	30(78.937%)	29(78.378%)
3) Serum HDL Cholesterol M=<40mg% F=<50mg%	18(47.368%)	23(62.162%)
4) Blood pressure Systolic=>130mmHg Diastolic=>85mmHg	35(92.105%) 31(81.578%)	29(78.378%) 24(64.864%)
5) Fasting plasma glucose =>100mg%	24(63.157%)	29(78.378%)

Out of 75 cases, 31 cases who were willing, were subjected to carotid artery Doppler study. Normal endothelial function was found in 16/31 cases. Endothelial dysfunction was found in 15/31 cases.

All individuals with syndrome x were given individualised as well as group health educative discussion, regarding the significance of the disease,

Table 3: Number of syndrome x cases with other risk factors by gender

Risk factors	Males	Females
Fly h/o DM	19	14
Fly h/o HTN	10	10
Fly h/o IHD	7	2
Fly h/o Obesity	1	0
Fly h/o CVD/PVD	1	0
High S. LDL Chol	17	9
High T.Chol	22	11
Gout	2	2
h/o smoking	4	0
h/o sedentary life style	9	15
h/o stress	3	5
CVD/PVD	0	0
BOH	-	1
LBW/Big baby	-	0
PIH/GDM	-	1
h/o Recurrent infections	0	0
h/o Alcohol intake	8	0

proper dietary changes and increased physical activity. Reduction in risk of development of atherosclerosis was aimed at by applying simple measures of weight reduction if obese, cessation of smoking, avoiding excess alcohol consumption, reduction of dietary intake of saturated fat and increasing consumption of fruit and vegetables, increased physical activity and appropriate medication (OHA's, anti hypertensives) when required. Antiplatelet agents and statins were added to those, who showed endothelial dysfunction(15/31 cases).

All the syndrome x individuals were followed up regularly and were encouraged to improve their participation. At the end of 2.5 years of regular follow up, all the criteria suggested by NCEP ATP III (2001) were repeated among syndrome x individuals, also patients who had shown endothelial dysfunction earlier, were subjected to repeat Carotid Artery Doppler and 75% of them had shown definite improvement/reversal in the carotid artery intima media thickness and reduction in the plaque size. All the syndrome x individuals showed shown significant improvement in various criteria (NCEP ATP III) such as improvement in blood pressure, waist circumference, Fasting Plasma Glucose & dyslipidaemia as shown in Table 4.

One of the major advantages with statins is that, they are well tolerated drugs with excellent safety record and large clinical trials have clearly demonstrated their beneficial effect on LDL-cholesterol levels. The pleotropic benefits of statins are also well known: 1) Improved endothelial function 2) Inhibition of LDL oxidation 3) Inhibition of release of cytokines. 4) Lowering of C-reactive protein levels 5) Stabilizing the plaque and preventing its rupture. 6) Inhibition of migration and proliferation of smooth muscle cells. 7) Inhibition of platelet activation 8) Inhibition of release of tissue factor. 9) Inhibition of matrix metalloproteinases. 10) No effect on fibrinogen levels and 11) Reduction in overall mortality).

The present study suggests identification of Syndome X individuals and consideration of statins and Antiplatelet agents in them, in addition to their usual management irrespective of whether every syndrome x individual is subjected to carotid artery Doppler or not.

HOT Study Group and U.S Physicians Health Study have concluded, that use of low dose aspirin for primary prevention in diabetic subjects.

Table 4: Number (percentage) of syndrome x individuals above the threshold of the variables of syndrome x at the beginning and after 2.5 years of follow up by gender.

Variables	Males		Females	
	Initial N = 38	>2.5yrs n=24	Initial N = 37	>2.5yrs n=26
Waist circumference M = >90 cm F = >80 cm	31/38 (81.578%)	14/24 (58.33%)	37/37 (100%)	19/26 (73.08%)
S triglycerides = > 150 mg%	30/38 (78.937%)	13/24 (54.166%)	29/37 (78.378%)	3/26 (11.5%)
Serum HDL Cholesterol M= <40mg% F = < 50mg%	18/38 (47.368%)	7/24 (29.166%)	23/37 (62.162%)	14/24 (58.33%)
Blood pressure Systolic =>130mmHg Diastolic = >85mmHg	 35/38 (92.105%) 31/38 (81.578%)	 10/24 (41.666%) 4/24 (16.666%)	 29/37 (78.378%) 24/37 (64.864%)	 9/26 (34.62%) 0/26 (0%)
Fasting plasma glucose =>100mg%	24/38 (63.157%)	15/24 (62.5%)	29/37 (78.378%)	5/26 (19.3%)

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Acknowledgements

Physicians: Dr. Yogesh Shejul and Dr.A.R.Kulkarni.
Department of Pathology.
Statistician Mr. Prashant.

Arterial Occlusion in Hyperhomocysteinaemia

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Introduction

Hyperhomocysteinaemia is linked with arteriosclerosis and thrombotic events. It is estimated that approximately 50% of patients with untreated hyperhomocysteinaemia will have major cardiovascular event, before the age of 30 yrs.

Case report

A 48 year old male patient, known case of hypertension on treatment, presented with complaints of cramping pain in right lower limb since last one month. Pain was absent on taking first step and appeared after exercise and which was relieved by rest. One month back cramping pain in calf muscle appeared after walking 500 meters which decreased to 200 meters over last one month. There was no history of smoking, diabetes, atrial fibrillation or previous myocardial infarction. No family history of similar complaint.

He also had a history of sudden episode of giddiness, double vision and loss of balance on walking some 8 years back. The investigation revealed that he had complete occlusion of bilateral vertebral artery, resulting in multiple cerebellar infarctions. He recovered from the same with conservative treatment.

On examination, both lower extremics were equally warm. He had right popliteal artery pulsation feeble, right dorsalis pedis absent and posterior tibial pulsation feeble. The left femoral, popliteal, dorsalis pedis, and posterior tibial arteries were well felt, right femoral was well felt. The ankle-brachial index on the right was 0.4 and 0.9 on the left.

Ultrasound examination of his lower extremities failed to reveal any DVT. He was investigated with right lower limb arterial doppler which showed complete block involving the mid and distal popliteal artery with collateral formation and evidence of diffuse narrowing and streaky blood flow in posterior tibial, anterior tibial and dorsalis pedis artery. His digital subtraction angiography was performed, which revealed complete occlusion of right popliteal artery with reformation of tibio-peroneal trunk through collateral and complete occlusion of distal anterior tibial artery.

His blood investigations were carried out to look for any prothombotic state, causing thrombosis of multiple arteries. Thrombophilia profile revealed that he had hyperhomocysteinaemia of moderate degree. Test results for mutations of prothrombin gene G 20210, protein C or S deficiency, and antithrombin III deficiency were negative. Serum homocysteine was 63 umol/L (normal 5 to 15 umol/L). Antibodies for anticardiolipin and the lupus anticoagulant were not detectable.

He was started on folic acid, vitamin B 12 and vitamin B 6 supplementation, for hyperhomocysteinaemia and he was managed conservatively with ecosprin and clopidogrel. No surgical intervention was contemplated in view of diffuse peripheral disease involving distal anterior tibial artery. He improved symptomatically with this treatment and was able to walk long distances without any pain.

Discussion

Hyperhomocysteinaemia is a blood disorder marked by an excess amount of the amino acid homocysteine in the blood stream. While healthy

levels of homocysteine in the blood are thought to help regulate metabolism and insulin absorption, high levels of this amino acid significantly strain the heart and damage the bones. Homocysteine levels are measured with a fasting blood test.

Aetiology and types of hyperhomocysteinaemia

There are two types of hyperhomocysteinemia: Primary and secondary:

1. Primary hyperhomocysteinaemia:

Due to inherited enzyme deficiency in the homocysteine pathways like:

- Cystathionine beta synthase (CBS) deficiency: It is the commonest deficiency, inherited as autosomal recessive trait, occurring in only 1 in 100,000 live births. It is characterized by dislocation of lens, skeletal deformities, mental retardation and premature atherosclerosis.
- 5, 10 methylene tetrahydrofolate reductase (MTHFR) deficiency: A mutation in the enzyme MTHFR is associated with hyperhomocysteinaemia, especially in the presence of low folic acid.
- Methylene tetrahydrofolate homocysteine methyl transferase deficiency.

2. Secondary hyperhomocysteinaemia

- Secondary causes of hyperhomocysteinemia include:
- Medications: Some people acquire hyperhomocysteinemia by taking certain medications, including anticonvulsants such as eptoin and carbamazepine, cyclosporine, methotrexate and theophylline.
- Nutritional deficiencies: Up to two-thirds of all hyperhomocysteinemia cases are due to nutritional deficiencies. These vitamins are folic acid (B8), cyanocobalamine (B12) and pyridoxine (B6), all vitamins that in turn help control levels of homocysteine.
- Malignancies: Malignancies of breast, ovary and pancreas. Acute lymphoblastic leukemia can also increase homocysteine levels significantly.
- Autoimmune diseases: Pernicious anemia, systemic lupus erythematosus.
- Other medical conditions: The presence of other, more serious underlying disorders can cause hyperhomocysteinemia. Liver impairment, kidney impairment, hypothyroidism can all cause hyperhomocysteinaemia

Measurement of plasma homocysteine

Normal total plasma homocysteine levels in the fasting state range from 5-15umol/L. depending upon these levels, Kang et al have classified hyperhomocysteinaemia into 3 types:

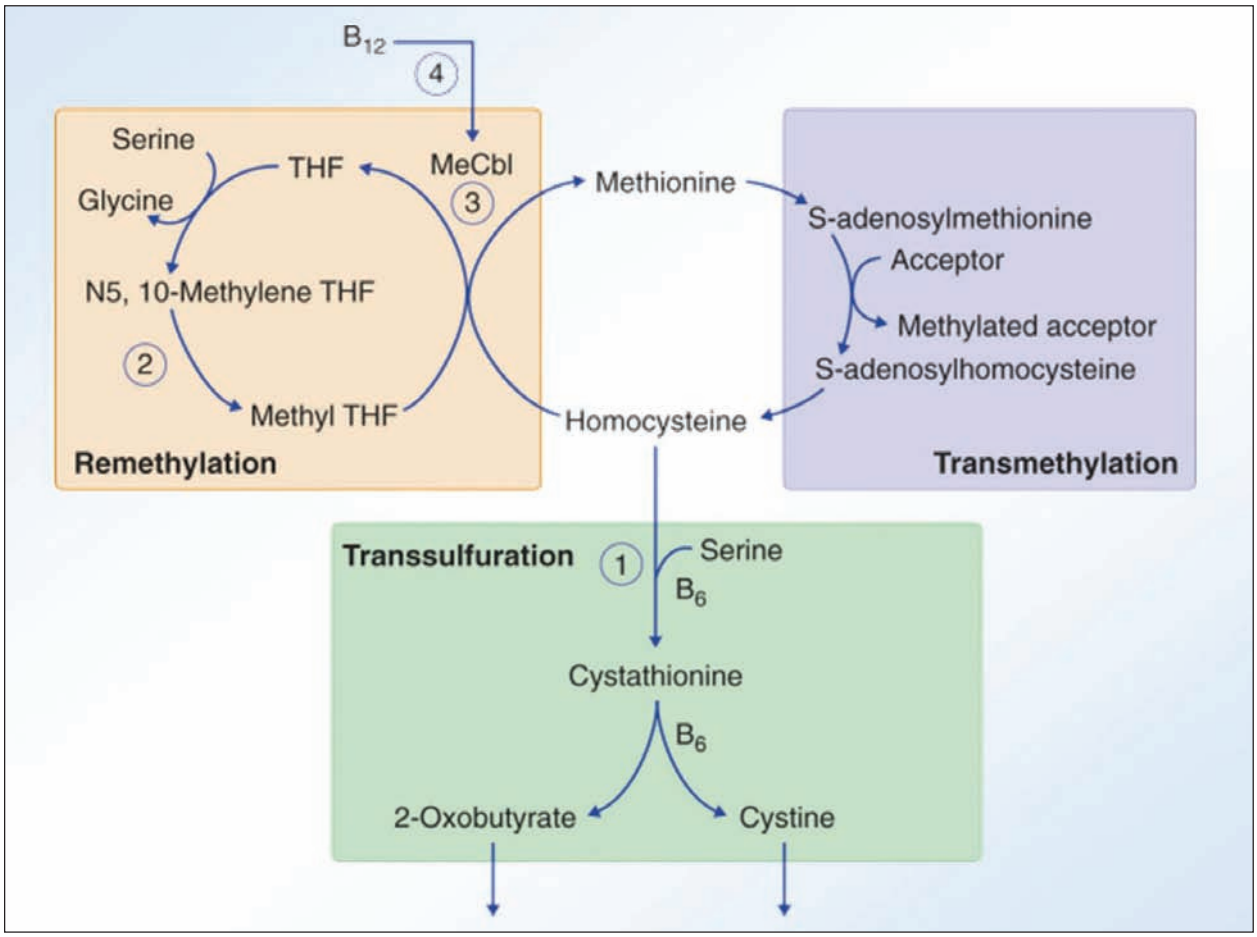
- Moderate risk: 16-30umol/L
- Intermediate risk: 31-100umol/L
- Severe risk : >100umol/L.

Approximately 5-7% of general population and 12-47% of patients with coronary and other vascular diseases have moderately elevated plasma levels of homocysteine.

Homocysteine is a sulphur-containing amino acid formed during the metabolism of methionine. Homocysteine is metabolized by one of two pathways: remethylation and transsulfuration. In the remethylation cycle, homocysteine is converted to methionine by the acquisition of a methyl group in a reaction catalyzed by methionine synthase. Vitamin B12 is an essential cofactor for methionine synthase, N5-methyl-tetrahydrofolate is the methyl donor in this reaction and N5,N10methylenetetrahydrofolate reductase (MTHFR), functions as a catalyst in the remethylation process.

Under conditions in which an excess of methionine is present or cysteine synthesis is required, homocysteine enters the transsulfuration pathway. In this pathway, homocysteine condenses with serine to form cystathionine, in a reaction catalyzed by the vitamin B6–dependent enzyme cystathionine-synthase.

Homocysteine metabolism



Pathways of homocysteine metabolism

The systems of transmethylation, remethylation, and transsulphuration are marked. Steps discussed are numbered:

(1) cystathionine β-synthase;

(2) methylene tetrahydrofolate reductase (MTHFR);

(3) methionine synthase and methyltransferase reductase;

(4) systems of cobalamin absorption, distribution, and reduction. B_6 = pyridoxine; B_{12} = cyanocobalamin/hydroxocobalamin; MeCbl = methylcobalamin; THF = tetrahydrofolate.

Cystathionine is subsequently hydrolyzed to form cysteine, which may in turn be incorporated into glutathione or further metabolized to sulfate and excreted in the urine.

The pathway, starting at methionine, progressing through homocysteine, and onwards to cysteine, is termed the transsulfuration pathway. Conversion of homocysteine back to methionine, catalyzed by MTHFR and methylcobalamin, is termed the remethylation pathway. A minor amount of remethylation takes place via an alternate route using betaine as the methyl donor.

The accumulation of homocysteine and its metabolites is caused by disruption of any of the 3 interrelated pathways of methionine metabolism—deficiency in the cystathionine B-synthase (CBS) enzyme, defective methylcobalamin synthesis, or abnormality in methylene tetrahydrofolate reductase (MTHFR).

Metabolism of homocysteine, thus requires three enzymes and three vitamins as cofactors. The enzymes

are methionine synthase and methylene tetrahydrofolate reductase both involved in the remethylation process and cofactors required for the process are folic acid and vitamin B12, two B complex vitamins. The third enzyme is cystathionine â synthase and the cofactor is vitamin - (pyridoxin) another B complex vitamin. These function in the transsulphuration pathway.

Mechanism of injury caused by hyperhomocysteinaemia

Several mechanisms have been suggested as the possible cause of accelerated vascular disease. These include (1) endothelial cell damage, (2) smooth muscle cell proliferation, (3) lipid peroxidation, (4) up-regulation of prothrombotic factors (XII and V), and (5) down-regulation of antithrombotic factors or endothelial-derived nitric oxide.

Treatment of hyperhomocysteinaemia

Treatment of hyperhomocysteinaemia varies with the underlying cause. However, vitamin supplementation with folic acid in combination with vitamins B6 and B12, is usually effective in reducing plasma levels of homocysteine. Vitamin supplementation (including folate, vitamin B_{12}, and vitamin B_6) effectively lower elevated plasma homocysteine concentrations to the normal range. Even if there is a genetic deficiency of any of the enzymes involved in homocysteine metabolism, supplementation with these vitamins can result in optimal functioning of the defective enzyme.

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Limb Body Wall Complex: A Rare Foetal Malformation: A Case Report

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Introduction

Limb Body Wall Complex (LBWC) is a rare foetal malformation, due to failure of development of body stalk. Limb body wall complex is a sporadic, lethal abnormality, a complex set of disruptive abnormalities consisting of two of the three following characteristics:

a) Abdominoschisis (anterior abdominal wall defect) and or thoracoschisis (thoracic wall defect)
b) Exencephaly (cranial defect) with/without facial clefts
c) Limb defects.

It is also associated with neural tube closure abnormalities like kyphoscoliosis of the spine, short umbilical cord, facial and cranial anomalies.

In the present case, the two diagnostic features were abdominoschisis and encephalocele with meningomyelocele, further supported by kyphoscoliosis of the spine, short umbilical cord with limb positional abnormalities.

This complex should be distinguished from other body wall defects including omphalocele and gastroschisis. The prognosis for Limb body wall complex is uniformly poor and most obstetricians consider it fatal. Other names associated with the syndrome are Body stalk anomaly or Cyllosomas.

Case Report

A 28 year old primigravida came for routine scan at 15 weeks of gestation to BARC hospital. On foetal ultrasound a rare dysmorphological anomaly was suspected and the diagnosis of limb body wall complex was made. This was then compared with the post-abortal gross examination findings and autopsy results.

USG findings

- Abdominal wall defect with herniating bowel loops.
- Posterior cranial vault defect with Exencephaly
- Kyphoscoliosis with maldeveloped spine
- Spinal defects with meningomyelocele
- Short umbilical cord
- Limb position abnormalities.

The limb bones were normal for length and development.

An ultrasound diagnosis of Limb body wall complex was made.

The couple was counseled and pregnancy was terminated. Subsequently feto-pathological examination which included gross examination and autopsy was performed. X-ray of the aborted foetus was taken.

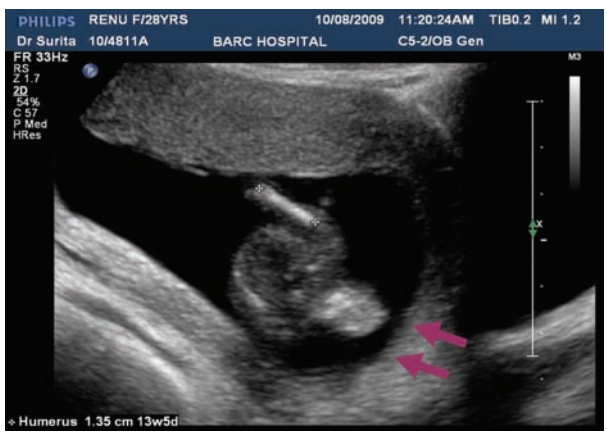


Fig. 1: Transabdominal ultrasound (Axial section) shows abdominal wall defect with herniated abdominal contents (arrows)

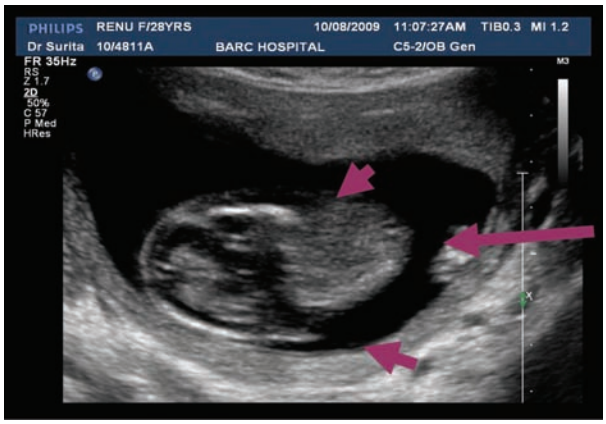


Fig. 2: Transabdominal ultrasound (axial section) shows a posterior cranial vault defect (small arrows) with herniation of brain parenchyma (long arrow)

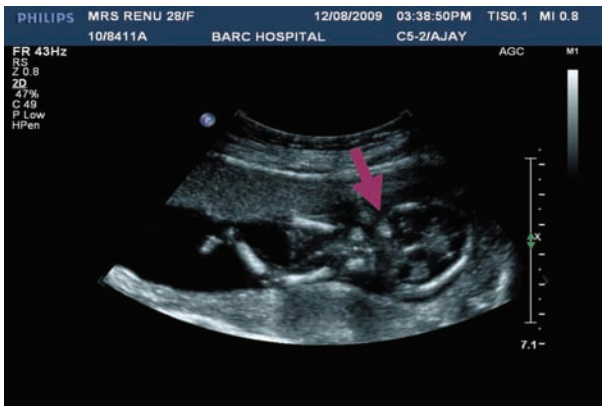


Fig. 3: Coronal Ultrasound section shows a short maldeveloped spine (arrow)

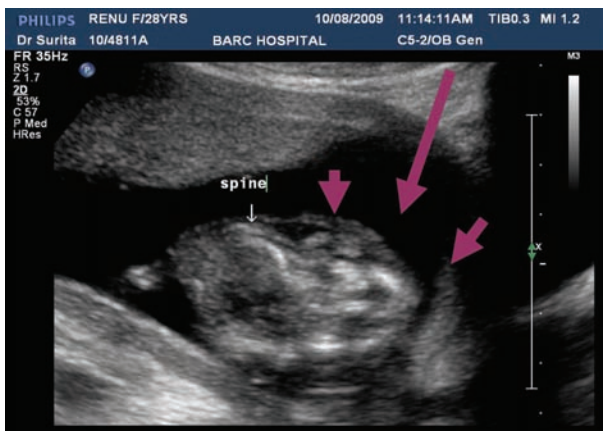


Fig. 4: Axial USG section shows spinal defect (small arrows) with meningomyelocele (long arrow) at the thoracic level.

Gross examination of aborted foetus

- Herniation of bowel loops through an abdominal defect with no covering membranes
- Large exencephaly with meningomyelocele in the cervico-thoracic region
- Maldeveloped spine
- Abnormally high position of lower limbs
- Short umbilical cord

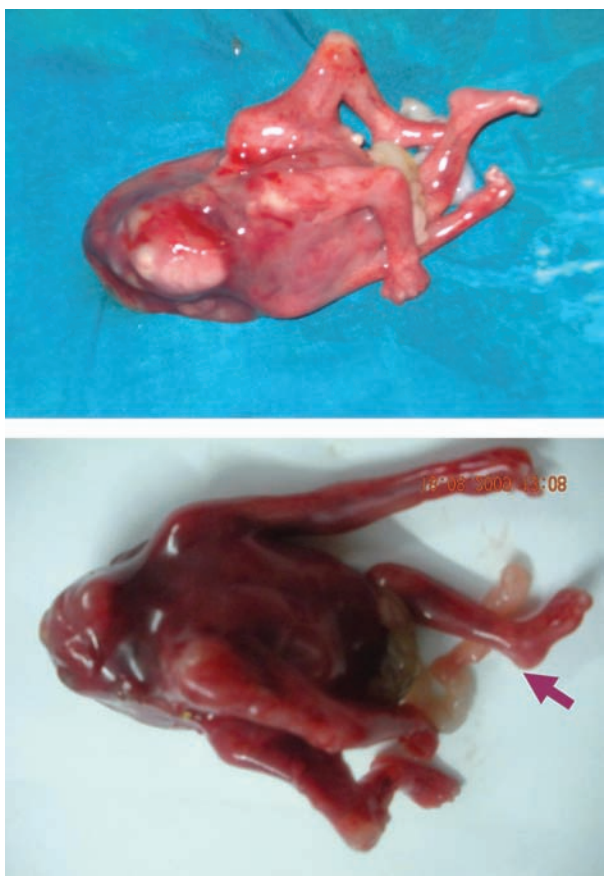
X ray findings (post abortal)

- ·Marked kyphoscoliosis
- ·Maldeveloped short spine
- ·Widening of pedicles
- ·Cranial defect

Autopsy findings

In addition to gross findings, autopsy revealed:

- Herniation of small bowel loops through the abdominal defect.
- Herniation of brain as well as spinal elements posteriorly through the cranial and spinal defects.



Figs. 5 & 6: Abortus reveals herniated bowel loops with short umbilical cord (arrow)

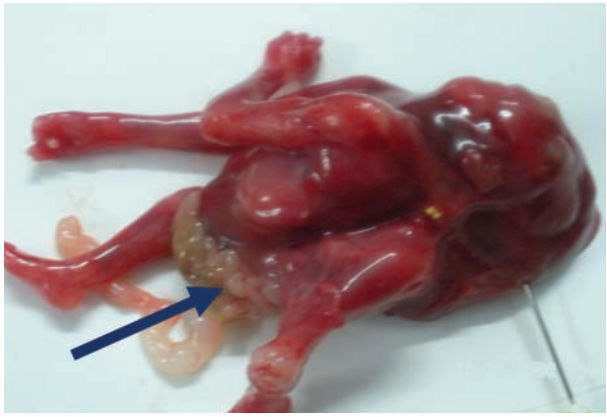


Fig. 7: Abortus shows herniated bowel loops (arrow) and short umbilical cord.Lower limbs are seen to arise from almost the same level as upper limbs.

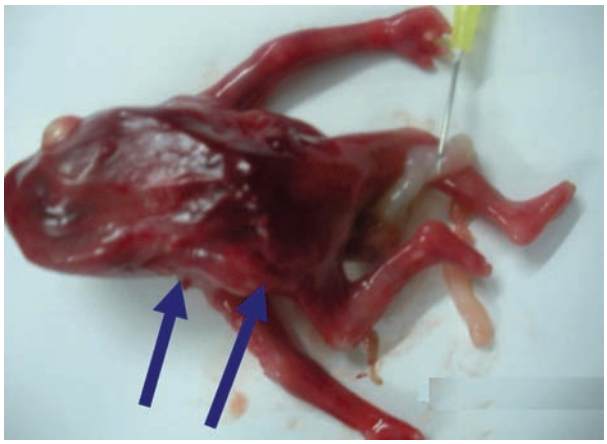


Fig. 8: Abortus from the dorsal aspect shows the encephalocele and meningomyelocele (arrows)

Discussion

The limb body wall complex is also known as the body-stalk syndrome. Limb body wall complex is a rare foetal malformation due to failure of development of body stalk.

[BODY STALK: The caudal end of the embryo is at first connected to the chorion by a band of mesoderm called the body-stalk. With the formation of the caudal fold the body-stalk assumes a ventral position. The function of the body-stalk is later replaced by the umbilical cord.]

It is a rare entity characterized by severe malformations. Most foetuses are aborted, either spontaneously or by medical means. Most of the remaining are stillborn. The etiology is unknown. No teratogen has been implicated and no genetic abnormality has been identified.

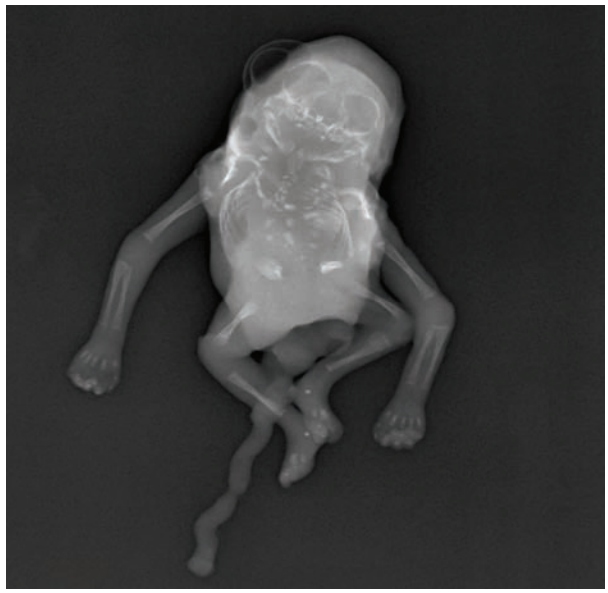


Fig. 9: Radiograph AP view of the Abortus

Epidemiology

The limb body wall complex syndrome is not always recognized; therefore, its incidence is difficult to estimate.

Kurosawa et al. estimated the incidence of occurance as approximately 0.21 to 0.31 cases per 10,000 births and found no connection to foetal sex, parental age or any other associated genetic anomalies.

Luehr et al. presented a series of 11 cases out of 33,286 births with a higher incidence: 3.3:10,000 births.

Diagnostic criteria

The diagnostic criteria for LBWC which are commonly employed are those originally set forth by Van Allen et al in 1987, i.e., the presence of two of the following three malformations:

a) Abdominoschisis and/or Thoracoschisis
b) Exencephaly with/without facial clefts
c) Limb defects.

But these criteria imply that an infant with encephalocele with facial clefts and limb defects can be considered as having LBWC and are hence disputed, because it would be inappropriate to make

the diagnosis in the absence of a body wall defect, which appears to be the primary anomaly.

In 1997, Martínez-Frías suggested that those cases with body wall defect be classified in its two main groups:

- Gastroschisis, for cases with an isolated (and usually small) body wall defect
- Body Wall Complex, for those cases with the body wall defect associated with other malformations, deformations or disruptions.

Russo et al. identified two distinct phenotypes of LBWC.

- Placentocranial adhesion phenotype associated with craniofacial defects, amniotic adhesions and amniotic band sequences.
- Placento-abdominal adhesion phenotype without craniofacial defects but with imperforate anus, urogenital abnormalities, spina bifida with/ without meningomyelocele, kyphoscoliosis, oligohydramnios, anomalies of the umbilical cord and short cord syndrome.

Anomalies which are associated with LBWC

Limb defects in LBWC include club foot, arthrogryposis, absent limb, single forearm bone, single lower leg bone, radial/ulnar hypoplasia. Cardiac malformations are truncus arteriosus, atrial septal defect, membranous VSD, hypoplastic right ventricle, ectopia cordis. Gastrointestinal anomalies seen are nonrotated intestine, intestinal atresia, anal atresia and Ladd's bands. Urogenital abnormalities reported are unilateral / bilateral absent kidney, pyelectasis, renal dysplasia; other anomalies are spina bifida with/ without meningomyelocele, kyphoscoliosis of spine, absent or short umbilical cord.

Short umbilical cord: causes and consequences

Umbilical cord growth occurs in response to tensile forces relating to intrauterine space availability and foetal movement during early development. A short umbilical cord is associated with early intrauterine constraint and with gross structural or functional limb defects that limits intrauterine movement.

Short umbilical cord has been shown to be associated with inadequate foetal descent, intrapartum haemorrhage and foetal heart rate abnormalities.

A normal karyotype in LBWC is characteristic. Very high maternal serum AFP levels are seen in LBWC.

There is no recurrence risk, but the condition is always fatal.

Theories on pathogenesis of LBWC

- *Germ disc defect* with early embryonic maldevelopment [Streeter, 1930; Herva et al., 1984; Bamforth, 1992]
- *Exogenic theory :* Primary rupture of the amnion caused by vascular or mechanical compression occurring between third and fifth weeks, leads to the formation of amniotic bands. Amniotic bands interrupt embryogenesis, are responsible for deformations and mutilations of the already formed foetal structures. [Torpin,1965]
- *Endogenous or vascular theory* suggests a vascular disruption/ischemic accident of the embryonic vessels between 4 and 6 weeks of gestation as the origin of this disease.

According to this theory, ischemia leads to a significant loss of foetal tissue, impairment of foetal development, abdominal wall disclosure, persistence of the extraembryonic coelom and adhesion of the amnion to the necrotized foetal parts. [Van Allen et all, 1987]

- Disturbance of the embryonic folding process, causing secondarily a disruption in the caudal and lateral folds. [Hartwig et al 1989, 1991].

Differential diagnosis of LBWC

- Numerous abdominal wall defects should be excluded before establishing the LBWC diagnosis with the following complex malformations being the most important:
- Amniotic band syndrome which is characterized by constriction or asymmetrical amputation with lymphedema beneath the level of constriction; asymmetrical cranio-facial malformation

(encephalocele, cleft palate); amniotic bands. Occasionally oligohydramnios with reduction of active foetal movements

- Pentallogy of Cantrell which is characterized by supra umbilical abdominal defect with Omphalocele, defect of the lower end of sternum, diaphragmatic defect, pericardial defect, ectopia cordis, and intracardiac anomalies
- Cloacal exstrophy which consists of infraumbilical wall defect, omphalocele, bladder exstrophy, anal imperforation as well as other renal, genital and skeletal anomalies.

It needs to be mentioned here that there is never bladder exstrophy in the limb body wall complex.

In the diagnostic approach to abdominal wall defects following factors are to be taken into consideration: are relationship of the cord insertion to the defect, presence of membranes, What organs are eviscerated?, characteristic of the herniated bowel, additional malformations .

Summary

Prenatal early diagnosis of Limb Body Wall Complex is possible by detection of very high maternal serum alpha-fetoprotein and by transvaginal ultrasound examination at the end of the first trimester. LBWC, a rare fetal malformation is almost uniformly lethal and must therefore be distinguished from other nonlethal fetal abdominal wall defects, such as gastroschisis or omphalocele. Early ultrasound diagnosis can thus be followed by medical termination of pregnancy.

Acknowledgement

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Infection Control Measures
The Bio-Medical Waste (Management and Handling) Rules, 1998

The Environment (Protection) Act, 1986 was published in the Gazette 1997 and reviewed by the Central Government which notified the rules for the management and handling of bio-medical waste. Some of the important points are discussed herewith:-

1. SHORT TITLE AND COMMENCEMENT

- These rules may be called the Bio-Medical Waste (Management and Handling) Rules, 1998.
- They have come into force on the date of their publication in the official Gazette.

2. APPLICATION

These rules apply to all persons who generate, collect, receive, store, transport, treat, dispose or handle bio-medical waste in any form.

3. DEFINITIONS (important ones)

- "Act" means the Environment (Protection) Act, 1986.
- "Authorised person" means an occupier or operator authorised by the prescribed authority to generate, collect, receive, store, transport, treat, dispose and / or handle bio-medical waste in accordance with these rules.
- "Bio-medical waste" means any waste, which is generated during the diagnosis, treatment or immunisation of human beings or animals or in research activities pertaining thereto or in the production or testing of biologicals and including categories mentioned in Schedule I;
- "Bio-medical waste treatment facility" means any facility wherein treatment disposal of bio-medical waste or processes incidental to such treatment or disposal is carried out.
- "Occupier" in relation to any institution generating bio-medical waste, which includes a hospital, nursing home, clinic dispensary, veterinary institution, animal house, pathological laboratory, blood bank.
- "Schedule" means schedule appended to these rules;

4. DUTY OF OCCUPIER

It shall be the duty of every occupier of an institution generating bio-medical waste which includes a hospital, nursing home, clinic, dispensary, veterinary institution, animal house, pathological laboratory, blood bank by whatever name called to take all steps to ensure that such waste is handled without any adverse effect to human health and the environment.

5. TREATMENT AND DISPOSAL

Bio-medical waste is treated and disposed of, in accordance with Schedule I, II and in compliance with the standards prescribed for disposal.

6. SEGREGATION, PACKAGING, TRANSPORTATION AND STORAGE

- Bio-medical waste is not to be mixed with other wastes.
- Bio-medical waste is to be segregated into containers/bags at the point of generation in accordance with Schedule II prior to its storage, transportation, treatment and disposal. The containers shall be labeled according to Schedule III.
- If a container is transported from the premises where bio-medical waste is generated to any waste treatment facility outside the premises, the container shall, apart from the label prescribed in Schedule III, also carry information prescribed in Schedule IV.
- No untreated bio-medical waste is to be kept stored beyond a period of 48 hours.

SCHEDULE I

[1][Waste Category No.]	Waste Category [1][Type]	Treatment and Disposal [1][Option +]
Category No.1	**Human Anatomical Waste** (human tissues, organs, body parts)	Incineration[@]/deep burial*
Category No.2	**Animal Waste**(animal tissues, organs, body parts carcasses, bleeding parts, fluid, blood and experimental animals used in research, waste generated by veterinary hospitals, colleges, discharge from hospitals, animal houses)	Incineration[@]/deep burial*
Category No.3	**Microbiology & Biotechnology Wastes**(Wastes from laboratory cultures, stocks or specimens of micro-organisms live or attenuated vaccines, human and animal cell culture used in research and infectious agents from research and industrial laboratories, wastes from production of biologicals, toxins, dishses and devices used for transfer of cultures)	local autoclaving/micro-waving/ incineration[@]
Category No.4	**Waste sharps** (needles, syringes, scalpels, blades, glass etc. that may cause puncture and cuts. This includes both used and unused sharps)	disinfection (chemical treatment [@@]/auto claving/ microwaving and multilation /shredding ##
Category No.5	**Discarded Medicines and Cytotoxic drugs** (wastes comprising of outdated, contaminated and discarded medicines)	incineration[@]/destruction and drugs disposal in secured landfills
Category No.6	[2][Soiled] **Waste** (Items contaminated with blood, and body fluids including cotton, dressings, soiled plaster casts, lines beddings, other material contaminated with blood)	incineration @autoclaving/ microwaving
Category No.7	**Solid Waste** (wastes generated from disposable items other than the waste [3][sharps] such as tubings, catheters, intravenous sets etc.)	disinfection by chemical treatment @@ autoclaving / microwaving and mutilation/shredding##
Category No.8	**Liquid Waste** (waste generated from laboratory and washing, cleaning, house-keeping and disinfecting activities)	disinfection by chemical treatment @@ and discharge into drains.
Category No.9	**Incineration Ash** (ash from incineration of any bio-medical waste)	disposal in municipal landfill
Category No.10	**Chemical Waste** (chemicals used in production of biologicals, chemicals used in disinfection, as insecticides etc.)	Chemical treatment @@ and discharge into drains for liquids and secured landfill for solids

@@ Chemicals treatment using at least 1% hypochlorite solution or any other equivalent chemical reagent. It must be ensured that chemical treatment ensures disinfection.

Mutilation/shredding must be such so as to prevent unauthorized reuse.

@ There will be no chemical pretreatment before incineration. Chlorinated plastics shall not be incinerated.

* Deep burial shall be an option available only in towns with population less than five lakhs and in rural areas.

+ Options given above are based on available technologies. Occupier/operator wishing to use other State-of-the-art technologies shall approach the Central Pollution Control Board to get the standards laid down to enable the prescribed authority to consider grant of authorisation].

SCHEDULE II

(See No. 6)

COLOUR CODING AND TYPE OF CONTAINER FOR DISPOSAL OF BIO-MEDICAL WASTES

Colour Coding	Type of Container	Waste Category	Treatment options as per Schedule I
Yellow	Plastic bag	Cat.1, Cat. 2,Cat.3 and Cat. 6	Incineration/deep burial
Red	Disinfected container/ plastic bag	Cat. 3, Cat.6 and Cat.7	Autoclaving/Microwaving Chemical Treatment
Blue/White translucent	Plastic bag/puncture proof container	Cat.4, Cat.7	Autoclaving/Microwaving/ Chemcial Treatment and destruction/shredding
Black	Plastic bag	Cat.5,Cat.9 and Gat.10 (Solid)	Disposal in secured landfill

Notes :

1. Colour coding of waste categories with multiple treatment options as defined in Schedule I, shall be selected depending on treatment option chosen, which shall be as specified in Schedule I.
2. Waste collection bags for waste types needing incineration shall not be made of chlorinated plastics.
3. Categories 8 and 10 (liquid) do not require containers/bags.
4. Category 3 if disinfected locally need not be put in containers/bags.

SCHEDULE III

(see Rule 6)

LABEL FOR BIO-MEDICAL WASTE CONTAINERS/BAGS

Note: Label shall be non-washable and prominently visible.

SCHEDULE IV

(see Rule 6)

LABEL FOR TRANSPORT OF BIO-MEDICAL WASTE CONTAINERS/BAGS

Day..... Month.....

Year .....

Date of generation.....

Waste category No.....

Waste Class

Waste description

Sender's Name & Address

Phone No.....

Telex No.....

Fax No.....

Contact Person.....

Receiver's Name & Address

Phone No.....

Telex No.....

Fax No.....

Contact Person.....

In case of emergency please contact :

Name & Address
Phone No.

Note : Label shall be non-washable and prominently visible.

CMEs conducted at BARC Hospital

January 2009 - December 2009

Date	Department	Topic
09 Jan. 2009	Orthopedics	Advances in management of Osteoporosis – Our Experience
15 Jan. 2009	ENT	Tracheostomy
23 Jan. 2009	Anaesthesia	Pre-Anaesthetic Evaluation of Patient with Cardiac Diseases for Non Cardiac Surgery
13 Feb. 2009	Psychiatry	Addiction Newer Approach
27 Feb. 2009	Pathology	Pre-Analytic Factors Affecting Quality Control In Pathology Laboratory
06 Mar. 2009	Infection Control Measures	Instrument Cleaning, Disinfection and Sterilization
13 Mar. 2009	Dispensary	A Study of Underweight Children in the Age Group of 1-10 Years
24 Apr. 2009	E N T	Allergic Rhinitis
08 May 2009	Paediatrics	Thalassemia and Iron Chelation in Thalassemia
12 May 2009	Infection Control Measures	Demonstration on Airflow by Infection Control
15 May 2009	Pathology	H1 N1 Influenza A
22 May 2009	Ophthalmology	Ocular Surface Disorders [Dry Eye]
02 June 2009	Anaesthesia	Types of Anaesthesia
12 June 2009	Radiology	Ectopic Pregnancy 2009
26 June 2009	Gynaecology & Obstetrics	Conservative Management of Ectopic Pregnancy
03 July 2009	Medical	Newer Insulin Analogue
10 July 2009	Medical	Early Diagnosis & Management of CKD
28 Aug. 2009	Orthopedics	Osteoarthritis of Knee
11 Sept. 2009	Paediatrics	Immunization Newer Guidelines
25 Sept. 2009	Psychiatry	Delirium
09 Oct. 2009	E N T	Epistaxis- An Overview
23 Oct. 2009	Ophthalmology	Retinal Vascular Occlusions
20 Nov. 2009	Medical	Dual Anti Platelet Therapy, Spine Pain & Instrumentation, Newer Techniques in Radiation Oncology
27 Nov. 2009	Radiology	Importance of Level – I Obstetric Scan
11 Dec. 2009	Anaesthesia	Fasting before Anaesthesia

BARC Hospital, Anushaktinagar, Mumbai

Edited & Published by;
Dr. K. Bhanumurthy
Head, Scientific Information Resource Division
Bhabha Atomic Research Centre, Trombay, Mumbai - 400 085, India
Editorial Support: Dr. (Ms.) S. C. Deokattey,
Computer Graphics & Layout: Mr. N. Kanagaraj, SIRD, BARC