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Oxidative Stress in the Development and Complications of Liver Cirrhosis

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Abstract

End stage-liver cirrhosis is associated with complications such as spontaneous bacterial peritonitis and hepatorenal syndrome. The generation of free radicals and biochemical alterations at the cellular and subcellular level in the intestine and kidney has been suggested to play an important role in these complications of cirrhosis. In addition, oxidative stress induced alterations in the intestinal cell surface glycosylation and qualitative, quantitative changes in the luminal bacterial flora might result in damage to the intestinal barrier and enhance bacterial adherence, resulting in translocation of bacteria into ascitic fluid leading to bacterial peritonitis. This review highlights the important role of oxygen free radicals involved in the different organ damage during and after development of experimental model of liver cirrhosis.

Key words: Spontaneous bacterial peritonitis, oxidative stress, ascites, carbon tetrachloride and thioacetamide

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Introduction

Oxidative stress elicited by reactive oxygen species has been suggested to play a role in the development of human liver disorders such as chronic viral hepatitis, alcoholic liver disease and primary biliary cirrhosis (1). Oxidative stress arises when there is an imbalance between radical-generating and radical–scavenging activity (2). Various studies have shown an increase in oxidative damage to lipids and proteins in serum and liver tissue as assessed by increased malondialdehyde and protein carbonyls content in patients with liver cirrhosis, which correlated with decreased antioxidant defense mechanisms seen in these patients (2-4). In addition, many etiological agents of fibrogenesis stimulate free radical generations (5), implicating these active species in the process. At the cellular level, it has also been shown that reactive oxygen species generated by the mitochondrial electron transport chain during experimental liver cirrhosis can damage subcellular compartments such as microsomes and mitochondria (6,7).

Liver cirrhosis is an advanced stage of progressive fibrosis due to chronic ongoing liver injury and is associated with two important cellular events: continuous hepatocyte loss and activation of hepatic stellate cells. Activation of hepatic stellate cells (HSC) is associated with increased extra cellular matrix production, which surrounds the regenerating hepatocytes (8,9). The development of liver fibrosis or cirrhosis is associated with increased morbidity and mortality(10). Most common etiologies of liver cirrhosis include alcoholism, chronic viral hepatitis, bile duct obstruction and autoimmune hepatitis. Cirrhosis can be induced in rats by administering various hepatotoxins such as carbon tetrachloride (CCl_4), thioacetamide (TAA), ethionine, dimethyl nitrosamine or through common bile duct ligation. Studies have suggested that histological and biochemical changes that develop in animals chronically treated with hepatotoxins like CCl_4 and TAA are comparable to human cirrhosis with different etiology.

Comparison of two different experimental models of liver cirrhosis:

Our studies using CCl_4 and TAA in experimental animals have shown that liver cirrhosis developed by 3 months of treatment and by 5 months, both the models were similar, where micro nodular cirrhosis was seen in CCl_4 and macro nodular form in TAA. Increased lipid and protein oxidation were

observed in mitochondria, peroxisomes and microsomes from the liver after carbon tetrachloride or thioacetamide treatment at different stages of cirrhosis development. Oxidative stress was more severe in animals treated with CCL_4 than thioacetamide. Mild oxidative stress was evident at 1 and 2 months of treatment and a significant increase was seen by 3 months of treatment with either compound, when compared to controls. This was accompanied by a decrease in antioxidant enzymes suggesting a role of oxygen free radicals in the early development of fibrosis and cirrhosis in both the models. The gradual increase in oxidative stress in the subcellular organelles such as mitochondria, peroxisomes and microsomes suggests that ROS are generated early during development of liver cirrhosis, though it is not yet clear if these active species are a result or cause of liver injury (11).

Complications of liver cirrhosis:

Mortality due to liver cirrhosis is predominantly due to development of complications of the disease. These include portal hypertension, spontaneous bacterial peritonitis and hepatorenal syndrome, which are major end-stage complications of patients with liver cirrhosis (12). Bacterial infection is responsible for up to one quarter of the deaths of patients with chronic liver disease (13). Spontaneous bacterial peritonitis (SBP) is a common and serious infection developing in cirrhotic patients, which appears as a consequence of impaired defense mechanisms against infection (14). SBP is defined as an abrupt onset of acute bacterial peritonitis without any apparent external or intra-abdominal focus of infection in patients with ascites caused by liver disease (15). Translocation of bacteria from the intestinal lumen to blood stream has been suggested to be involved in the pathogenesis of SBP (16). End-stage liver cirrhosis also results in marked alterations in systemic circulation and renal function (17). The progressive reduction in the renal blood flow and glomerular filtration rate along with impaired ability to excrete sodium and water lead to ascites in cirrhosis (18, 19). The worsening of renal function and sodium and water retention in cirrhosis not only correlates with the elevation in plasma vasopressin, renin, aldosterone, and nor epinephrine but also with the degree of portal hypertension (20, 21).

Small intestinal alterations in animal model of liver cirrhosis:

Intestinal bacterial overgrowth, altered permeability of the mucosa and

deficiencies in host immune defenses has been implicated in the development of SBP. Clinical and experimental evidence indicate that translocation of bacteria from the intestinal lumen into mesenteric lymph node and to the blood stream is directly involved in the pathogenesis of SBP (16, 22). The gastrointestinal tract is affected during cirrhosis and mucosal abnormalities secondary to portal hypertension may exist (23). Our studies have demonstrated oxidative stress in the intestinal mucosa after CCl_4 induced-liver cirrhosis, where the activity of xanthine oxidase, an important source of free radicals in the small intestine is elevated (24,25). Mucosal alterations attributed to oxidative stress, including disturbed enterocyte mitochondrial function and increased lipid peroxidation of mitochondria and brush border membranes were also evident

Bacterial adherence is accomplished by specific adhesins on the outer surface of bacteria that attach to receptors containing sugars such as sialic acid, hexose, fucose and amino sugars on the surface of the epithelial cells (26,27). We showed that liver cirrhosis results in increased sugar content of both intestinal brush border membrane and surfactant layers. These changes could be the result of oxidative stress, since free radicals are known to modulate the glycosyl transferase or glycosidases which might in turn alter the glycosylation pattern (28,29). This was accompanied by changes in bacterial flora in the gut, which showed increased bacterial hydrophobicity and adherence onto the epithelial cells. This might facilitate translocation across the mucosa, resulting in complications such as SBP. The increased adherence of bacteria from cirrhotic rats was sugar specific, since prior addition of sugars such as galactose, fucose and mannose in the *in vitro* system, inhibits and reverses the adherence property of the bacteria. The role of oxidative stress was further confirmed by the inhibition of xanthine oxidase using sodium tungstate and antioxidant supplementation using vitamin E, which offered significant protection against the oxidative stress, changes in brush border membrane sugar content and bacterial adherence (30).

Spontaneous bacterial peritonitis (SBP) results in oxidative and nitrosative stress in ascitic fluid:

As an extension of the animal studies, we also carried out experiments on ascitic fluid from patients with SBP. Ascitic fluid from cirrhotic patients with and without SBP were examined for oxidative and nitrosative stress. The

first line defenses against infection in the peritoneal cavity are neutrophils and macrophages (31). Human neutrophils contain inducible nitric oxide synthase which produces nitric oxide (NO). Myeloperoxidase, an enzyme present in the neutrophils can produce oxygen free radicals. Simultaneous presence of both NO and ROS can result in the formation of reactive nitrogen species such as peroxynitrite (32), which is a highly reactive anion and contributes to microbial killing (33). In patients with SBP, increased nitrate and increased oxidative stress parameters such as malondialdehyde, protein carbonyls were evident in ascitic fluid as compared to cirrhotic controls without SBP. Treatment of these patients with the antibiotic cefotaxime for 48 hours reversed the oxidative stress and decreased the nitrate levels in ascitic fluid. Thus the measurement of oxidative stress markers and nitrate levels in the ascitic fluid would probably be useful in the diagnosis of SBP and in the follow-up after antibiotic treatment (34).

Renal damage in animal models of liver cirrhosis:

As mentioned earlier, the hepatorenal syndrome is a complication of cirrhosis, and it has been demonstrated that cirrhosis with ascites is associated with impaired renal function accompanied by sodium and water retention. Inhibition of nitric oxide synthases significantly improves renal function in cirrhotic animals, suggesting a role for nitric oxide in renal pathophysiological events induced by decompensated cirrhosis (35). Our ultra structural studies demonstrated mitochondrial dilation and glomerular epithelial swelling in the kidneys of cirrhotic animals. Platelets and cytoplasmic blebs were also present in the kidneys of rats treated with both carbon tetrachloride and thioacetamide for a period of 3 months. It was observed that cirrhosis results in oxidative stress in the kidney as seen by increased lipid peroxidation and protein oxidation parameters, accompanied by decreased anti-oxidant status. Liver cirrhosis also affected the function of renal mitochondria, as seen by decreased respiratory control ratio, swelling of mitochondria and altered calcium flux across the mitochondrial membranes. Increased lipid peroxidation and changes in lipid composition were also evident in the renal brush border membranes, with compromised transport across these membranes. What could be the sources for these oxygen free radicals? The uncoupling of

mitochondrial respiration and oxidative phosphorylation in the kidney, indicated by our experimental evidence, might result in enhanced generation of super oxide anions which may be involved in the damage. In addition, free radicals generated from infiltrating neutrophils have been proposed to be a major cause for cellular damage associated with many chronic inflammatory diseases (36). We observed an increased activity of myeloperoxidase in the kidney of cirrhotic animals indicating neutrophil infiltration, suggesting that this could be an additional source for reactive oxygen species in this context. The changes seen in the kidney *per se*, as well as in renal mitochondria and renal brush border membrane were minimal after the first and second months of treatment with these hepatotoxins and were prominent only after full development of liver cirrhosis, which occurred after 3 months of treatment (37). These ultrastructural and biochemical changes seen in the kidney might play an important role in the development of complications of cirrhosis such as hepatorenal syndrome.

Conclusion

The studies presented here have demonstrated the involvement of oxygen free radicals in the development of liver cirrhosis and its complications in experimental animals. Oxidative stress was seen in the liver of rats treated with carbon tetrachloride and thioacetamide for a period of 3 months which continued to increase till five months of treatment. Liver cirrhosis also resulted in oxidative stress in the intestine due to the generation of super oxide from xanthine oxidase and mitochondrial dysfunction resulting in protein and lipid oxidation. Development of liver cirrhosis resulted in alterations in the intestinal cell surface glycosylation and qualitative and quantitative changes in the luminal bacteria. These alterations in the glycocalyx on the surface of the intestinal epithelium may result in damage to the intestinal barrier and enhance bacterial adherence, resulting in translocation of bacteria into ascitic fluid leading to bacterial peritonitis. In addition, patients with spontaneous bacterial peritonitis showed evidence of oxidative stress and increased nitrate level in the ascitic fluid which was decreased with antibiotic treatment. Oxidative stress was also observed in the kidney in experimental liver cirrhosis which was evident at 3 months of treatment and continued to increase till 5 months, suggesting a role for liver cirrhosis induced oxidative stress in the renal damage seen in cirrhosis (Fig 1).

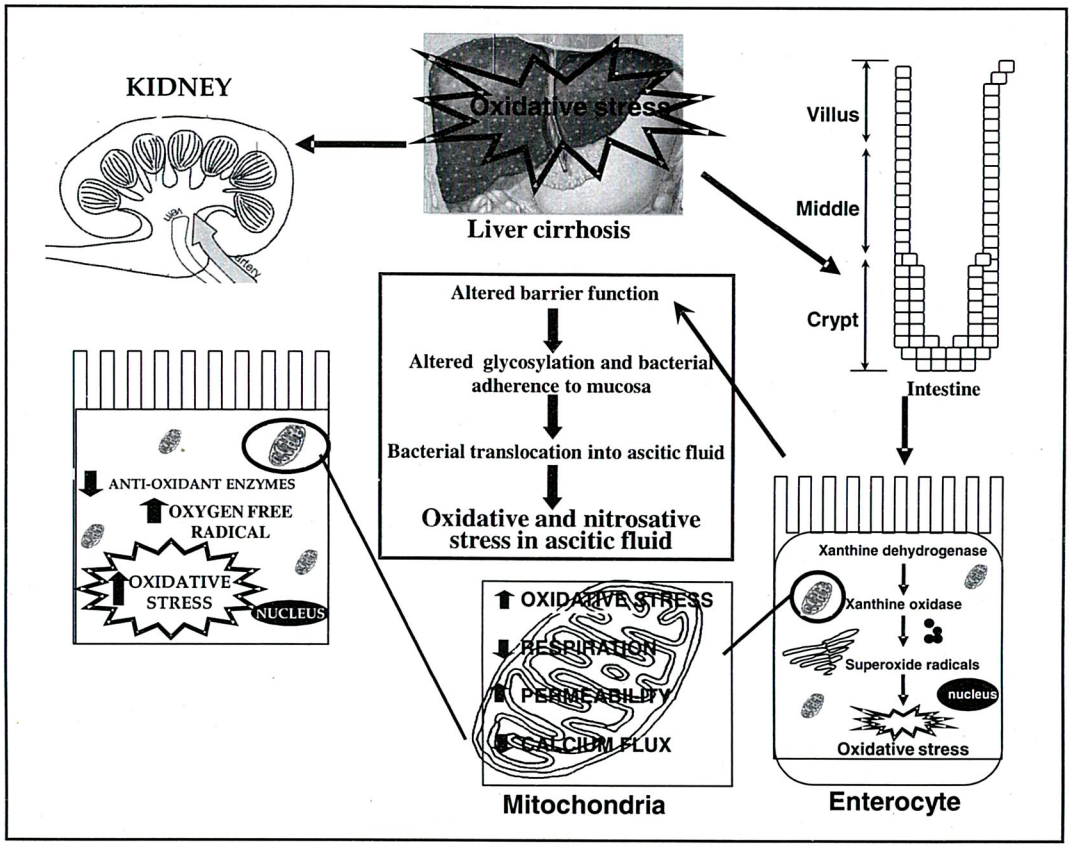

Figure 1: Events in the complications of liver cirrhosis. Oxidative stress in the intestine following liver cirrhos is due to the possible conversion of xanthine dehydrogenase into xanthine oxidase leading to increased super oxide generation and brush border membrane damage. These changes together result in altered gut barrier function followed by increased glycosylation and increased bacterial adherence into mucosa, and thereby translocation of bacteria into ascitic fluid. On the other hand, increased oxygen free radicals and decreased antioxidant enzymes were also observed in the kidney. In addition to this, mitochondrial dysfunction was evident in both intestine and kidney following liver cirrhosis.

References

1. Aboutwerat A, Pemberton PW, Smith A *et al.* (2003). Oxidant stress is a significant feature of primary biliary cirrhosis. *Biochim Biophys Acta* **1637**: 142-150.

2. Ljubuncic P, Tanne Z and Bomzon A. (2000). Evidence of a systemic phenomenon for oxidative stress in cholestatic liver disease. *Gut* **47**: 710-716.

3. Trevisani F, Caraceni P, Simoncini M *et al.* (2002). Evidence of oxidative imbalance in long-term liver transplant patients. *Dig Liver Dis* **34**: 279-284.

4. Yamamoto Y, Yamashita S, Fujisawa A, Kokura S and Yoshikawa T. (1998). Oxidative stress in patients with hepatitis, cirrhosis, and hepatoma evaluated by plasma antioxidants. *Biochem Biophys Res Commun* **247**: 166-170.

5. Poli G and Parola M. (1997). Oxidative damage and fibrogenesis. *Free Radic Biol Med* **22**: 287-305.

6. Krahenbuhl S and Reichen J. (1992). Adaptation of mitochondrial metabolism in liver cirrhosis. Different strategies to maintain a vital function. *Scand J Gastroenterol Suppl* **193**: 90-96.

7. Muller D, Sommer M, Kretzschmar M *et al.* (1991). Lipid peroxidation in thioacetamide-induced macronodular rat liver cirrhosis. *Arch Toxicol* **65**: 199-203.

8. Parola M and Robino G. (2001). Oxidative stress-related molecules and liver fibrosis. *J Hepatol* **35**: 297-306.

9. Wright MC, Issa R, Smart DE *et al.* (2001). Gliotoxin stimulates the apoptosis of human and rat hepatic stellate cells and enhances the resolution of liver fibrosis in rats. *Gastroenterology* **121**: 685-698.

10. Anselmi K, Subbotin VM, Nemoto E and Gandhi CR. (2002). Accelerated reversal of carbon tetrachloride-induced cirrhosis in rats by the endothelin receptor antagonist TAK-044. *J Gastroenterol Hepatol* **17**: 589-597.

11. Natarajan SK, Thomas S, Ramamoorthy P *et al.* (2006). Oxidative stress in the development of liver cirrhosis: a comparison of two different experimental models. *J Gastroenterol Hepatol* **21**: 947-957.

12. Heidelbaugh JJ and Sherbondy M. (2006). Cirrhosis and chronic liver failure: part II. Complications and treatment. *Am Fam Physician* **74**: 767-776.

13. Wyke RJ. (1987). Problems of bacterial infection in patients with liver disease. *Gut* **28**: 623-641.

14. Chang CS, Yang SS, Kao CH, Yeh HZ and Chen GH. (2001). Small intestinal bacterial overgrowth versus antimicrobial capacity in patients with spontaneous bacterial peritonitis. *Scand J Gastroenterol* **36**: 92-96.

15. Correia JP and Conn HO. (1975). Spontaneous bacterial peritonitis in cirrhosis: endemic or epidemic? *Med Clin North Am* **59**: 963-981.

16. Yeh DC, Wu CC, Ho WM *et al.* (2003). Bacterial translocation after cirrhotic liver resection: a clinical

investigation of 181 patients. *J Surg Res* **111**: 209-214.

17. Schrier RW, Arroyo V, Bernardi M, Epstein M, Henriksen JH and Rodes J. (1988). Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology* **8**: 1151-1157.

18. Bataller R, Gines P, Arroyo V and Rodes J. (2000). Hepatorenal syndrome. *Clin Liver Dis* **4**: 487-507.

19. Arroyo V and Colmenero J. (2003). Ascites and hepatorenal syndrome in cirrhosis: pathophysiological basis of therapy and current management. *J Hepatol* **38 Suppl 1**: S69-89.

20. Forrest E, Jalan R and Hayes P. (1996). Review article: renal circulatory changes in cirrhosis—pathogenesis and therapeutic prospects. *Aliment Pharmacol Ther.* **10**: 219-231.

21. Ming Z, Fan YJ, Yang X and Lautt WW. (2005). Blockade of intrahepatic adenosine receptors improves urine excretion in cirrhotic rats induced by thioacetamide. *J Hepatol* **42**: 680-686.

22. Guarner C, Runyon BA, Young S, Heck M and Sheikh MY. (1997). Intestinal bacterial overgrowth and bacterial translocation in cirrhotic rats with ascites. *J Hepatol* **26**: 1372-1378.

23. Llovet JM, Bartoli R, Planas R *et al.* (1994). Bacterial translocation in cirrhotic rats. Its role in the development of spontaneous bacterial peritonitis. *Gut* **35**: 1648-1652.

24. Ramachandran A and Balasubramanian KA. (2001). Intestinal dysfunction in liver cirrhosis: Its role in spontaneous bacterial peritonitis. *J Gastroenterol Hepatol* **16**: 607-612.

25. Ramachandran A, Prabhu R, Thomas S, Reddy JB, Pulimood A and Balasubramanian KA. (2002). Intestinal mucosal alterations in experimental cirrhosis in the rat: role of oxygen free radicals. *Hepatology* **35**: 622-629.

26. Prabhu R and Balasubramanian KA. (2004). Altered glycosylation of surfactant and brush border membrane of the small intestine in response to surgical manipulation. *J Surg Res* **117**: 272-282.

27. Delmotte P, Degroote S, Lafitte JJ, Lamblin G, Perini JM and Roussel P. (2002). Tumor necrosis factor alpha increases the expression of glycosyltransferases and sulfotransferases responsible for the biosynthesis of sialylated and/or sulfated Lewis x epitopes in the

human bronchial mucosa. *J Biol Chem* **277**: 424-431.

28. Basivireddy J, Jacob M, Ramamoorthy P and Balasubramanian KA. (2005). Alterations in the intestinal glycocalyx and bacterial flora in response to oral indomethacin. *Int J Biochem Cell Biol* **37**: 2321-2332.
29. Tatsumi Y, Kodama T, Kashima K, Ohkuma S and Kuriyama K. (1992). Inhibition of gastric glucosamine synthetase activity by oxygen radicals: a possible cause of decreased mucosal protective capacity. *Jpn J Pharmacol* **58**: 391-398.
30. Natarajan SK, Ramamoorthy P, Thomas S *et al.* (2006). Intestinal mucosal alterations in rats with carbon tetrachloride-induced cirrhosis: changes in glycosylation and luminal bacteria. *Hepatology* **43**: 837-846.
31. DeLeo FR, Allen LA, Apicella M and Nauseef WM. (1999). NADPH oxidase activation and assembly during phagocytosis. *J Immunol* **163**: 6732-6740.
32. Beckman JS and Koppenol WH. (1996). Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol* **271**: C1424-1437.
33. Vazquez-Torres A, Jones-Carson J, Mastroeni P, Ischiropoulos H and Fang FC. (2000). Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. I. Effects on microbial killing by activated peritoneal macrophages in vitro. *J Exp Med* **192**: 227-236.
34. Natarajan SK, Mukhopadhya A, Ramachandran A, Amalanathan S, Kurian G and Balasubramanian KA. (2007). Spontaneous bacterial peritonitis results in oxidative and nitrosative stress in ascitic fluid. *J Gastroenterol Hepatol* **22**: 177-181.
35. Martin PY, Ohara M, Gines P *et al.* (1998). Nitric oxide synthase (NOS) inhibition for one week improves renal sodium and water excretion in cirrhotic rats with ascites. *J Clin Invest* **101**: 235-242.
36. Galkina E and Ley K. (2006). Leukocyte recruitment and vascular injury in diabetic nephropathy. *J Am Soc Nephrol* **17**: 368-377.
37. Natarajan SK, Basivireddy J, Ramachandran A *et al.* (2006). Renal damage in experimentally-induced cirrhosis in rats: Role of oxygen free radicals. *Hepatology* **43**: 1248-1256.

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Disaster Management

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Abstract

Disasters are a matter of global concern. The death toll from natural disasters is about 250,000 per year. In India too, loss of life due to floods and earthquakes is considerably high. The term disaster refers to a natural or a man made event in combination with its damaging effects, which results in affecting a number of people large enough to disrupt the normal course of emergency and healthcare services. The common denominators are hazard, risk and vulnerability. A large number of classifications are available based on origin/ cause, whether natural or man made. Disaster planning cannot prevent disasters but the effects can be minimized by appropriate plans and preparedness. Disaster management is an intensive exercise involving inputs from local, national and international sources, requiring coordination in the management of a disaster preparedness plan and public participation in restoring normalcy with good speed.

Key words: Disaster management, natural disasters, man made disasters, disaster response, disaster prediction.

Introduction

Disasters are common occurrences all over the world. India is no exception; floods affect over nine million hectares annually. Fifty six percent of land mass is vulnerable to seismic activity of

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varying degrees and 5700 km of coastline is prone to severe cyclones, with extensive damage of life and habitat. The economic loss is considerable and rehabilitation even more costly. Besides natural disasters we also have man made disasters like transport accidents, railway accidents, social tensions and bomb blasts.

Globally the toll of death and damages in natural disasters is increasing and the cost to global economy is estimated to be 50 billion US dollars per year. One-third of the cost is for predicting, prevention and mitigating whereas two-thirds of the cost is due to direct damage. A death toll globally is about 2.5 lakh patients per year. Even in India loss of life due to floods, earthquakes is considerably high leading to a mechanical disaster.

The word disaster is French in origin, being derived from two words, "des" (meaning bad or evil) and "aster" (meaning star), literally meaning "bad or evil star".

The disasters have been defined in various ways on the basis of degree of physical impact of the event, magnitude, disruption of public safety, disproportion of resources and in terms of special efforts required.

Definitions:

World Health Organization (WHO): Sudden ecologic phenomenon of sufficient magnitude to require external existence.

American College of Emergency Physicians (ACEP): Any community or regional event that disrupts community functions and activities and threatens or causes concern for the lives, health and property of the citizens.

Humberside County Council, UK: Major incident arising with little or no warning causing or threatening death or serious injury to or rendering homeless, such numbers of persons in excess of those which can be dealt with by the public services operating under normal procedures and which calls for the special mobilization and organization of these services.

In common parlance disaster means disruption of such magnitude that organization, infrastructure and resources are overwhelmed. Destruction is so large that it exceeds the capacity of a community to adjust and requires assistance from out side. As per the Disaster Management Act 2005, 'Disaster' means a catastrophe, mishap, calamity or grave occurrence in any area, arising from natural or man made causes, or by accident or negligence which results in substantial loss of life or human suffering or damage to, or destruction of property, or damage to, or degradation of environment, and

is of such a nature or magnitude as to be beyond the coping capacity of the community of the affected area.

Generally speaking the term disaster refers to a natural or a man made event in combination with its damaging effects, which results in a number large enough to disrupt the normal course of emergency and healthcare services.

The Common Denominators are hazard, risk and vulnerability

Hazard refers to the natural event itself. Risk refers to the probability that a particular system/ population will be affected by hazards. Vulnerability refers to the susceptibility of a population/ system to the effects of the hazard.

Classifying Disasters

A large number of classifications are available based on origin/ cause whether natural or man made. They could also be based on the source or on the onset or on the anticipatory response.

Classification Based on Origin/ Cause

The classification based on origin or cause can be a natural disaster like earthquake, Tsunami, volcanic eruption or it can be due to natural phenomena on earth surface like landslides and avalanches. Other classifications based on origin or the cause could be due to natural disasters like cyclones, typhoons, hurricanes, hailstorms, sandstorms, floods, and droughts. It could also be biological in term of epidemics and locust invasion.

Disaster could also be classified on the basis of man made disasters like those caused by warfare, civil disturbances (riots and demonstrations) or caused by accidents like drowning, building collapse, explosion etc and vehicular accidents. Other classification could be based on the source – metereological (storms, hurricanes, cyclones, droughts, cold spells), topological (floods, landslides and avalanches), telluric and tectonic (earthquakes, volcanic eruptions) and accidents (explosions, fires).

Classification Based on Response

Level 1: Local emergency response personnel/ organization are able to contain & respond effectively.

Level 2: Requires regional efforts and aid from surrounding country/ community.

Level 3: Local resources overwhelmed; Needs international assistance/ aid.

Various authors have described the response of disaster:

Powell & Rayner Model (1952) describes disaster as a series of temporal stages. It proposes a set of

processes by which an occurrence is defined as a disaster:

- Stage 0 – Pre Disaster Scenario
- Stage 1 – Warning Phase
- Stage 2 – Threat Phase
- Stage 3 – Impact Phase
- Stage 4 – Inventory Phase
- Stage 5 – Rescue Phase
- Stage 6 – Remedy Phase
- Stage 7 – Recovery Phase

Skeet (1977) suggested five phases of disaster:

1. Warning phase
2. Period of Impact
3. Rescue phase
4. Relief phase
5. Rehabilitation phase

The phases of disaster could have also been described as:

1. Inter-disaster period.
2. Pre-impact stage.
3. Disaster phase – Disaster strikes the community and can have the following phases: stage of isolation, rescue, relief, phases of temporary shelter and stage of rehabilitation.

Process of disaster

The geographic divisions of area concerned with disaster were conceived in order to classify the arising problems and to help manage them. Three major divisions in vogue are, impact area, filter area and community aid area; *impact area* is where disaster has struck causing damage; *filter area* is undamaged zone surrounding the impact area from where immediate aid by community starts; the *community aid area* is immediately outside the filter area from where the organized rescue and relief flows.

Principles of disaster planning

Disaster Management means a planned and systematic approach towards understanding and solving problems in the wake of disasters. The disaster planning cannot prevent disasters but its effects could be minimized by appropriate plans and preparedness. Some of the general principles of disaster planning are universal and can be applied in all the situations. The foremost requirement is that it should be a continuous process, it should reduce the unknown in a problematic situation, and it should be based on valid knowledge. Other important facts are that it should evoke appropriate action, focus on general principles, and it must be tested. The disaster plans and preparedness to deal with disaster situation are necessary for every community particularly the health care system with its critical component, the hospitals which are to

be prepared consistently to mobilize all their facilities for maximum use. Realistic, well-rehearsed and properly coordinated disaster plan executed by well-trained system is essential to meet the challenge of disasters. The key issues in disaster management are communication, coordination and control. Important issues in predisaster management are prediction, prevention, planning and preparedness. The critical issue when disaster event occurs is the immediate response, rescue, relief and rehabilitation.

Disaster Management

Disaster has been conceptualized as a process with different temporal phases, where different information and action is required. The disaster management can be divided into 5 phases.

The basis of disaster management is *disaster prevention, disaster mitigation* (that is warning systems, evacuation plans etc.) and *disaster preparedness*. This is followed by *recovery phase* which is the process by which the community is assisted in returning to the level of functioning prior to the disaster. The best response of the disaster management is by way of disaster measures taken prior to, during and fcllowing the disaster, with an aim to save life, to protect property and the immediate damage caused by the disaster.

The activation of *disaster response* is by warning, notification, organization of command and scene assessment, which is followed by implementation (search and rescue, extrication, triage, stabilisation and transportation).

In other words it actually entails taking control and management of scene of disaster. Ultimately the management also includes reconstruction and rehabilitation and entails returning the community to the level prior to the disaster.

Disaster Response

Disaster response can be local/ regional, national or even international. The local response can be provided by the state administration, local government, NGOs and from the local community itself.

The national response depends upon the policy and resource availability within the country. Disaster prone nations, where disasters are common, have designated task forces, expert teams and disaster management committees to out line the responsibility as well as the resources needed for mitigation of the disaster.

International organizations have also been of help in the immediate aftermath of the disaster by providing supplies and equipments relating to communication, generators, food, clothing as well as shelters.

The essentials of disaster management are clear cut disaster management policies, adequate legislation, describing the responsibilities of the relief organization and preparedness. The preparedness and response depend upon risk and hazard assessment and a having vulnerability analysis. Technology plays a major role in data collection, assessment and development of hazard maps (1).

The other parameters of disaster management are disaster prediction and warning. These depend upon the meteorological, hydrological and seismological data providing warning of cyclone and floods in the coastal areas and the use of modern sophisticated techniques like aerial photography, satellite imaging and other remote sensing systems (2).

The other essential aspects of disaster management are awareness of the resource plans and training of manpower in managerial, technical and coordination skills. Finally, essentials of disaster management should also include public awareness and education and conducting periodic disaster drills at the local, district and state levels.

Disaster management in India

Overview

The contingency action plan for natural calamities was mooted by ministry of agriculture in India in 1990. It was a brief policy statement and response plan. It was mainly relief oriented. The mitigation and preparedness was not given much importance. In 2001 National Disaster Commission was constituted after the earthquake in Gujarat on 26th January, 2001. The National Disaster policy was outlined which emphasized the need for creation of a separate body for designing, implementation and development of National Disaster Management plan (3). It was envisaged that in disaster management the responsibility would be shared between the centre and the states wherein state would provide basic responsibility of relief and rescue and the centre would play a supportive role providing information, financial, technical and material support. The National Disaster Management Authority (NDMA), established in 2005, is headed by the Prime Minister. The NDMA has the following responsibilities:

1. Lay down policies on disaster management;
2. Approve the National Plan;
3. Approve plans prepared by the Ministries or Departments of the Government of India in accordance with the National Plan;
4. Lay down guidelines to be followed by the State Authorities in drawing up the State Plan;
5. Lay down guidelines to be followed by the different Ministries or

Departments of the Government of India.

6. Coordinate the enforcement and implementation of the policy and plan for disaster management;
7. Provide support to other countries affected by major disasters.
8. Take measures for the prevention of disaster, or the mitigation, or preparedness and capacity building for dealing with the threatening disaster situation or disaster.
9. Lay down broad policies and guidelines for the functioning of the National Institute of Disaster Management.

Under this the National Disaster Response Force was established with a multidisciplinary high tech skilled force to deal with all types of disasters. Under this scheme eight battalions were formed from different organizations i.e., Border Security Force (BSF), Central Reserve Police Force (CRPF), Central Industrial Security Force (CISF) and Indo Tibetan Border Police Force (ITBP).

National Institute of Disaster Management (NIDM) was established as the centre for human resource development in the areas of disaster mitigation and response for training, research, data collection, capacity development and documentation. There has been a *paradigm shift* in the management of disaster from relief orientation to preparedness.

The various ministerial responsibilities have been identified for different types of disasters as given in Table 1.

- Earthquakes and Tsunami	Ministry of Home Affairs (MHA)/ Ministry of Earth Sciences/ Indian Meteorological Department (IMD)
- Floods	MHA/ Ministry of Water Resources/ Central Water Commission (CWC)
- Cyclones	MHA/ Ministry of Earth Sciences/IMD
- Drought	Ministry of Agriculture
- Biological Disasters	Ministry of Health and Family Welfare
- Chemical Disasters	Ministry of Environment & Forests
- Nuclear Disasters	Ministry of Atomic Energy
- Air Accidents	Ministry of Civil Aviation
- Railway Accidents	Ministry of Railways

The impact on hospitals in case of disasters can be direct or indirect. In case of a natural disaster the hospitals and their services can be severely affected if the hospital lies in the direct impact area and the health infrastructure can be damaged. In case of a man made disaster like bomb blast, the hospitals are not affected and they can play a stellar role in management of the disaster. The indirect impact of hospitals during the disaster can be requirement of networking of the hospitals, sending rescue teams to the impact areas and the various measures for improvisation like operation theatres, emergency rooms and training for mechanical, biological and nuclear warfare.

In conclusion, it needs to be reiterated that disaster management is an intensive exercise involving inputs from all sources – local, national and international, requiring coordination in the management for a disaster preparedness plan and involvement of the public in participation and restoring normalcy with good speed.

References

1. Carter W Nick, Disaster Management. A Disaster Management Handbook. Asian Development Bank, Manila, 1991.
2. United Nations Disaster Relief Coordination. Disaster Prevention and Mitigation. Volume 10. Public Information Aspect, UN, New York, 1979.
3. National Disaster Management Authority, Govt. of India. National Disaster Management Guidelines – Medical Preparedness and Mass Casualty Management. Oct 2007.

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Networking in Disaster Management

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Abstract

Effective health relief management depends on anticipating disasters before they arise and identifying likely problems. Disaster preparedness must be undertaken long ahead through situational analysis, operational planning, training and networking. Networking is essential for effective and efficient disaster management. The main requisites and components of networking and the essentials for the effective networking are discussed. Networking helps in inventory analysis of existing resources, knowledge augmentation of involved agencies and optimal utilization of resources. For efficient networking, the essential prerequisites are well established Standing Operative Procedures (SOP), predetermined allocation of resources and duties under various contingencies, regular training and rehearsals and efficient network linkages for communication, transport, materials and manpower.

Key words: disaster management, networking, communication, tele-medicine, mobile teams, systems approach.

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Introduction

Disaster, whether man made or natural, may occur at any place or time. The ubiquitous characteristic feature in disaster management is disproportion between requirements and resources, which call for efficient and effective mobilization of ones own resources and external assistance. In order to achieve optimization of resources it is essential that in disaster management, clubbing of resources is done. Effective and efficient disaster management requires a multi institutional approach.

NETWORKING

Networking may be termed as linking up for augmentation/ optimization of available resources, which may be in the form of information, materials and/or manpower. Figure 1 depicts the dimensions of networking.

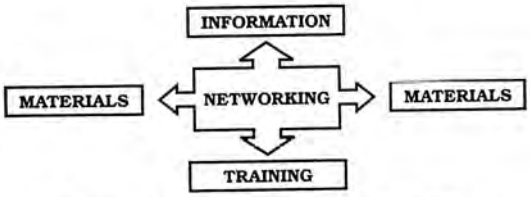

Figure 1: Dimensions of networking

Advantages of Networking

The following are the advantages:

Aids in Inventory Analysis of Existing Resources

It is essential in the networking process to have information about the existing resources. These resources may be in the form of materials or manpower. An analysis of these will aid in assessing capabilities/limitations. Steps may be undertaken for enhancing the capabilities and reducing the constraints. Inventory analysis is of utility for future planning e.g., during an inventory analysis it may be revealed that some hospital equipment may need phasing out, so rather than waiting for the time when they become nonfunctional, a planned phasing out may be implemented.

Aids in Knowledge Augmentation

By sharing information, the networked hospitals have access to each other's inventories / facilities. This may be gainfully employed by them to critically examine their respective materials and manpower resources. Resources and/or facilities may accordingly be phased out / augmented.

Aids in Optimal Utilization of Resources

If there is no networking, then in a disaster scenario resources are requisitioned from nearby hospitals. This may result in duplication / non availability. Networking alleviates this problem, since affected health care institutions have detailed information regarding the availability of resource of all the networked health care institutions.

Two-way system

Unlike the referral system which is a one –way system, networking is a two-way system. It thus offers a more effective communication system and an overall productive output.

It is a Systems Approach

A system is defined as an aggregation of objective/personnel/ organizations limited by some form of regular interaction or interdependence. Disaster management requires sharing of inputs to maximize output.

Requisites of Networking

Standard Operating Procedures (SOPs)

Chronological and functional operative steps must be formulated, adopted and executed by the networked hospitals. The SOPs must incorporate the following:

- When to activate networking?
- Who all are authorized to activate networking?
- Whom to contact? Names, appointments, telephone nos. pager nos. fax nos./ e-mail/web site addresses.
- Budgetary allocations
- Billing – who pays?
- Intra and inter sectoral coordination
- Triage allocation - standardized protocols should be enunciated which will facilitate transfers, receipt and management of casualties.

Allocation of Resources

After detailed inventory/ situation analysis and assessment of health care institutions capabilities, resources allocation under various contingencies should be planned. The following resources should be planned on regional/ networked areas/facilities:

- Mobile medical assistance teams
- Mobile vehicles for pharmaceutical and other supplies
- Mobile blood banks
- Air ambulances.

Forecasting methods such as multivariate analysis and simulation models may be utilized for resource allocation.

Allocation of Duties

Duties of manpower and health care institutions which are networking should be clearly defined. The duties should include the contingency when the disaster is in the area of jurisdiction of the health care institution or when it has to provide aid to another health care institution under whose jurisdiction the disaster has occurred.

Training

Training is an important aspect for the successful implementation of the disaster management. In networking, since various internal and external agencies are involved, training is an essential requisite. Triage allocation criteria, communication operations, pre hospital care principles and practice must also be incorporated in the training schedule. Prompt response, when disaster occurs, should be the cornerstone on which emphasis must be given during the various phases of training.

Rehearsals

Rehearsals are essential to train, practice and to know the existence of lacunae if any, in the networking process. Apart from full scale rehearsals, drills may also be carried out in the form of mini drills or by simulation.

Universal Sensitization

When disaster occurs apart from health care institutions, other agencies and community directly or indirectly get affected. It is essential to make the community aware of the importance of the disaster plan, its execution and the role of the various functionaries, agencies and institutions.

Effective and Efficient Communication

To achieve effectivity and efficiency in networking, communication becomes an inevitable essential ingredient. Effective communication involves simultaneous, identical understanding of the content and intent of the sender and receiver. The barriers of communication *viz.* delay, distortion and dilution may be easily surmounted by modern day communication systems of mobile phones, fax, e-mail, and the internet.

Communication and Networking

Communication is a vital component of DM. In addition, communication systems are also vulnerable to failure during disasters, thus it is important to develop strategies to protect these systems and to make them more disaster resilient.

(a) All hospitals will be connected with the Integrated Ambulance Network, QRMTs/MFRs and various emergency functionaries of ICS through a dedicated communication network. All hospitals will have a intra-/inter-hospital horizontal network. Dedicated telephone numbers will be made available to hospitals. The network will also be integrated with

police, fire and other helpline services.

(b) A specialized communication network will also include tele-medicine. The Indian Space Research Organization (ISRO) has established two-way video conferencing facility using specific satellite bandwidth at both sides i.e., provider and user end at some places. The village Resource Centre (VRC) of ISRO will also be utilized. BSNL is also expanding its broadband connectivity to taluka level. These communication systems will be utilized for tele-medicine during disaster.

(c) Mobile tele-health is another concept of telemedicine that can be used for disasters by putting diagnostic equipment and Information Communication Technology (ICT) together on a vehicle to get connectivity between the disaster site and advanced medical institutes where such connectivity already exists. Such systems may be placed in known disaster prone areas or could be moved at the onset of disasters. Such systems will be developed at regional levels.

(d) The development of GIS-based statistical data, population indices and demographic data by ground level documentation and data management during mass casualty events. This data will be made available to the surveillance teams.

(e) A single emergency toll-free telephone number will be introduced for the entire country.

(f) Mechanism to check the redundancy of data, voice communication and security of the communication network will be developed.

(g) Radio-frequency based systems to locate and manage resources and inventory will be allocated at specific locations.

(h) All the communication systems within the medical-set up will be linked to the national communication network.

The essential parameters which must be considered while networking are listed in the appendix.

Components of Networking

Networking has multifaceted and stratified dimension. The following need to be gainfully networked.

Communication

Effective communication is the key to productive networking. Link up should be established by all feasible

modes *viz.* telephones, mobile phones, fax, e-mail, wireless (the police service are effectively linked by this mode) LAN (Local Area Networking), WAN (Wide Area Networking) and Internet (a dedicated website for the networked health care institution is preferable). GH Net (Global Health Network), CHUDRC (Global Health Unit for Disaster and Relief Coordination) are existing and proposed net sites respectively which may be gainfully utilized.

Transport

Various modes of transport of the participating health care institutions need to be networked. Modalities for their requisition need to be spelt out in the SOPs. In management of disasters apart from ambulances other vehicles like general vehicles, trucks or tractors may have to be incorporated.

Rail and/or air transport may have to be resorted to for providing medical aid or evacuation of casualties. The existing medical resources of the Indian Railway Services especially the Accident Relief Medical Equipment (ARME-1 and ARME-2) available at the medical establishments, at regular places, throughout the railway network need to be utilized as and when required. It is preferable for the networked health care institutions to have dedicated / on call air assistance in the form of air ambulance / transport planes.

Materials

Expendable pharmaceutical products, hospital equipment and appliances form components of material networking. Inventory analysis aids in planning for procurement, requisitioning during disasters and in referral of patients, e.g. if CT scan services are available in a net worked hospital, head injuries casualty evacuation from the disaster site may be done to this center rather than follow the routine evacuation chain to the next echelon of health care even though it may not have CT scan facilities.

Blood is another important life saving product required in disaster management. Location, blood donors list from all sources including civil, private, NGOs and Armed Forces health care establishments should be available with the networked hospitals and they should have the capability to transport blood products to the hospitals where it is required.

Manpower

Specialist medical officers, paramedics and other health care workers all form important links in the networking process. Mobile teams / quick reaction teams (QRTs) of various categories of health personnel must be earmarked and ready to move at short notice, to the site or to the health care institution as and when they are requisitioned.

Fig. 2 depicts suggested flow of patients in the networked hospitals. Triage is carried out at the nearest health care institution. The stable patients after being administered first aid may either be discharged or evacuated to another networked hospitals. Unstable patients after resuscitation may be retained or referred depending upon the availabity or absence of facilities.

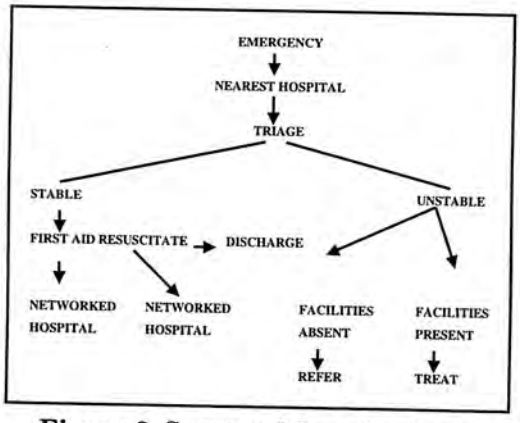

Figure 2: Suggested flow of patients in networked hospitals

Fig. 3 depicts the networking of health care institutions at various levels. Primary health centers (PHCs), community health centers (CHCs) and district hospitals may be networked with each other and also with a least one super specialty centre. Coordination has also to be established with the local administration including the district magistrate, police, fire brigade. Liaison has also to be maintained with local private hospitals, local Armed Forces medical establishments, Red Cross and other NGOs.

Communication networking between the PHCs, CHCs and district hospitals may be by telephones, mobile phones and fax. District hospitals may be interlinked with each other and other specialist centers through WAN or Internet. A dedicated website will be of significant utility for institutions having Internet facilities.

ESSENTIAL FOR EFFECTIVE NETWORKING

Situation, Hazard and Capability Analysis

A critical analysis of these would be of immense help. Situation analysis should include.

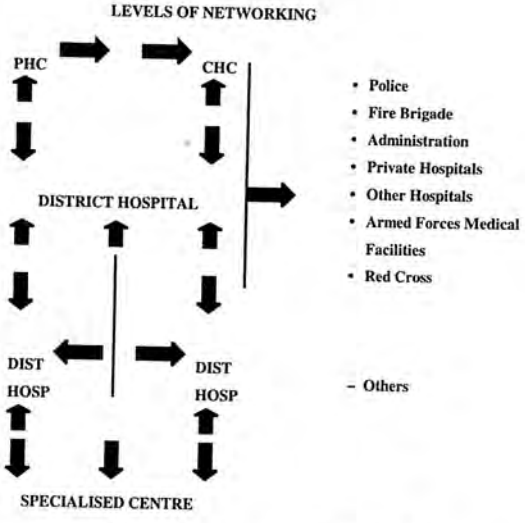

Figure 3: Networking of health care institutions at various levels

- Organizational set up of health care services in the region
- Topography, population and population density of the area
- Administrative set up details at village, tehsil, district and state levels
- Disaster mapping of the area. These include industrial sites, earthquake prone areas, disaster epidemic zones and so on.

Inventory Analysis of Manpower, Equipment and other Pharmaceutical Products

This will aid in planning, assigning duties and responsibilities, patient referrals, updating and in mobilization of resources.

Duties and Responsibilities

Duties and responsibilities of the networked personnel, agencies and health care institution should be clearly delineated.

Standing Operative Procedures

SOPs should be scientifically formulated, adopted and reviewed.

Training

Training is termed as a process by means of which the aptitudes, skills and capabilities of individuals to perform specific jobs are increased. Hence selection criteria for disaster management should include an aptitude for life saving, skill for quick response, flexibility and the capability to work under stress.

Coordination

Coordination between the networking health care institutions and other agencies like the police, fire brigade are essential for successfully implementing networking.

Mobile Teams

Mobility is essential in networking. Mobile surgical teams, blood banks will provide efficiency to the networking process.

Rehearsals

Frequent rehearsals are very essential. To save time and resources mini drills and simulations should be carried out.

Communication

Communication in networking should be a process of meaningful interaction amongst human beings. Computer / satellite assisted communication should be gainfully utilized.

Chain of Networking

The networking process should cover the entire sphere of operations. There should be intra and inter

networking of districts states and the nation.

Conclusion

Disaster is characterized by suddenness of occurrence, vastness of damage, loss of life, property, disruption of communication and so on. Effective health relief management depends on anticipating disasters before they arise and identifying likely problems / needs. Consequently, systems approach should be formulated and adopted. Disaster preparedness must be undertaken long ahead through situational analysis, operational planning, training and networking. In a developing country like India networking of hospitals in disaster management will enhance capabilities, optimize output and increase effectivity. Networking will be a significant contributing factor in achievement of the disaster planning aim of "Saving as many lives as possible by provision of best possible health care". It has been rightly said **"the best managed disaster is the disaster which is prevented."**

References

1. National Disaster Management Authority, Govt. of India. National Disaster Management Guidelines – Medical Preparedness and Mass Casualty Management. Oct 2007.
2. Tanenbaum AS (1999). Computer Networks, Prentice-Hall of India Private Ltd, New Delhi.
3. Bates RJ (1994). Wireless Networked Communications, N.York, McGraw Hill.

APPENDIX
Points for considerations while networking

1. Which are the networking hospitals?
   (a) Have the hospitals intended for networking been identified?
   (b) Have inventory analysis of manpower and materials been carried out?
   (c) Are action cards available with all concerned?
   (d) Are the strengths and constraints of those hospitals known?
   (e) Has a formal or informal agreement been made?
2. When to network?
   (a) What is the number and types of casualties which necessitate activating networking with other hospitals?
   (b) Is a disaster code available?
3. What are the modalities of networking?
   (a) Are the names, telephones and addresses of the key personnel of the net-working hospitals available?
   (b) How will they be contacted: telephone, fax, wireless, radio/ e-mail, LAN, WAN, web site?
   (c) Who is authorized to contact?
   (d) Who will be contacted?
   (e) Is the formulation of command nucleus defined?
4. Transport and Movement
   (a) Which of the vehicles will be used to shift casualties?
   (b) Will vehicles from other agencies be requisitioned?
   (c) Other modes of transport to be requisitioned – rail, air.
5. Records
   (a) What type of records will be maintained to monitor the referrals?
   (b) What mechanism will be devised to inform the relatives, press and VIPs?
6. Orientation of staff
   (a) How will the staff of health care institution be made aware of the networking plan?
   (b) How will the participating hospital staff be oriented to the plan?
7. Administrative and financial issues?
   (a) Does the plan require administrative approvals?

(b) Have separate budgetary provisions been made?

(c) Has financial sanction been obtained?

8. Intersectoral coordination

(a) Have other agencies (e.g. district collector , police, fire services, telephone exchange, NGOs, military medical establishments) been identified and assistance arranged?

9. Testing and Review of Networking?

(a) How will the networking be tested and reviewed?

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Nuclear, Biological and Chemical Warfare/ Terrorism - Medical Preparedness for Effective Planning

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Abstract

The threat of nuclear, biological and chemical (NBC) warfare, as a full- fledged warfare or as a localized terrorist attack, is a distinct possibility in the current international political and military scenario. Dissemination of information and training of all concerned i.e. army, general public, fire brigade, police and other organizations must be carried out before a NBC disaster occurs. Comprehensive planning is required for the protection and management of the resulting casualties and the complex nature of the injuries sustained.

Key words: nuclear warfare, chemical warfare, biological warfare, nuclear medicine, bioterrorism.

Introduction

In the current international, political and military scenario Nuclear, Biological and Chemical (NBC) warfare/ threat has assumed a realistic dimension. The effects of NBC warfare will be far reaching. It is pertinent to note that a NBC warfare may not occur

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only as a full fledged warfare. It may take shape of a localized terrorist attack.

Salient Features in Medical Treatment of NBC Casualties

The following are the salient features: (1)

- The magnitude of casualties will generally far outstrip the medical and other resources.
- Specialized ambulances will be required for casualty evacuation.
- Health care facilities including equipment and health care workers may themselves suffer significant damage.
- The responders may require Personal Protective Equipment (PPE).
- Triage protocol to be followed in a NBC scenario needs to be different from that followed during other disasters.
- Casualty evacuation has to be done separately for contaminated and non contaminated cases.
- Significant number of casualties would be burn casualties.
- An integrated multidisciplinary program on capacity development through education, training and critical infrastructure development is required
- There will be significant psychological stress disorder cases including those of Acute Stress Disorder and Post Traumatic Stress Disorder.
- Community should have the knowledge of the effects of NBC agents with basic emergency precautions and preventive measures.
- The medical preparedness for Chemical, Biological, Radiological and Nuclear (CBRN) management necessitates development of SOPs for CBRN management at the incident site for triage, personal protection decontamination, resuscitation, casualty evacuation followed by treatment of exposed victims at the hospital level. The critical infrastructure for medical management includes CBRN casualty treatment centers /wards and training facilities for specialist response to deal with covert CBRN attacks.

Status in India

Government of India in recognition of the importance of disaster management as a national policy had set up a High Powered Committee (HPC) in August 1999 and a National Committee on Disaster Management (DM) after the Gujarat Earthquake for

making recommendations on the preparation of DM plans and for suggesting effective mitigation mechanism. Recommendations of the HPC laid the foundation for DM framework in India. Indian Ocean Tsunami acted as the catalyst and the Government of India took a defining step in the legislative history of the country by enacting DM Act 2005. Of the eight Battalions of National Disaster Relief Force four are specially trained to manage NBC disasters. (2)

Effects of Nuclear Explosion

Blast, thermal and ionizing radiation are the different mechanisms which are responsible for nuclear casualties. Immediately after a nuclear explosion temperature may go upto 106 degree centigrade and pressure upto 105 atmospheres. The energy of nuclear explosion is released in the form of blast (fifty percent) heat (thirty five percent) and nuclear radiation (fifteen percent). The flash of light of a nuclear burst is followed by a ball of fire. Gamma rays and neutrons are emitted from the ball of fire. This is followed by pressure wave called the shock wave which travels at supersonic speed. This wave after striking the earth is reflected back. The reflected and the incident waves fuse together to form the Mach Wave. There is an initial and residual ionizing radiation on after detonation of nuclear weapon. The initial radiation is released within the first minute after detonation whereas the delayed radiation includes the local fall out due to debris which reaches the ground within few days and global fallout which enters the atmospheric circulation and falls on the earth surface after months or even years. The various types of ionizing radiations released are neutrons, alpha and beta particles, gamma and X-rays.

Protective measures

The various protective measures which should be known to all are as follows:

- Not to look at the flash of a nuclear explosion. To prevent retinal burns and flash blindness, eyes should be closed.
- If outdoors, protection from the blast should be taken by lying down immediately on the ground.
- Protection from heat should be taken in underground shelters with overhead cover.
- Avoidance of entry into contaminated areas.
- Not consume suspected food, water, fruit, milk and vegetables.
- Personal cleanliness.
- Decontamination of personnel and equipment at decontamination centres.

- Periodic medical review of personnel who have been exposed to small doses of radiation.

Essentials for effectivity

Organizational Set-up

To deal effectively with NBC warfare casualties, planning for an appropriate organizational set up is an essential requisite. Special centers capable of providing protection from the NBC effects must be catered for, at district, state and national levels. VIPs key personnel, equipment including communication set up, should have provision for taking protective shelter in the event of a NBC strike.

Burn Centers

A large number of victims of a NBC warfare will be burn casualties. Burns may be caused by 'flash', fire or beta particles after a nuclear fallout. Specialized burn centers should be functional to cater for the management of these casualties.

Decontamination Centers

As an operative guideline all casualties of a nuclear warfare must be regarded as radiation victims unless otherwise proved. Monitoring and decontamination of the residual radioactive contamination should be done. The injured must pass through a decontaminated center into protected area and be screened at a reception area. Washing bathing and laundry facilities should be provided at decontamination centers.

Additional Support

Augmented support both in terms of medical and administrative will be required for management of NBC casualties. Planning must be done for air lifting of manpower and other resources.

Training

Training in NBC warfare related events must be done for maximum number of civil and military personnel. Use of dosimeters, general precautions and measures in case of a NBC attack should be known.

Triage Protocol

Nuclear explosion casualties require a triage protocol which is different than that for other casualties. The most seriously injured with multiple injuries and those with irradiation dosage significant should get the last priority. The first priority should be given to casualties who have a reasonable chance of survival, if treated.

Radiological and Nuclear Emergencies

Nuclear Medicine is a branch of medicine and medical imaging that uses

radioisotopes in diagnostic and therapeutic measures. Nuclear energy has numerous widespread applications in the field of industry, medicine, agriculture and research. Because of these widespread applications, the availability of radioactive sources has become easy. While their radioactive strength is in itself a deterrent to pilferage, they do have the potential of being stolen and used in a Radiological Dispersal Device (RDD) or Improvised Nuclear Device (IND) (2).

CBRN Stores

Specified hospitals for CBRN treatment should stock all the drugs, decorporation agents and other specialized items for treatment of CBRN casualties (2). They will include:

- Growth factors, colony stimulating factors, and other radiation recovery agents are very useful for restitution of the immune system.
- Antidotes are required to be procured to neutralize chemical effects. Antidotes required for nerve agents are physostigmine, obidoxime, atropine and pyridostigmine. Vesicants may require dimercaprol, sodium thiosulphate while cyanide based agents may require dicobalt edetate.
- Biological agents require antibiotics and vaccines. Recombinant protective antigen vaccine and anthrax immuno-globulin for anthrax, recombinant F1-V antigen vaccine for plague and vaccines for Q fever, tularemia, botulism, viral hemorrhagic fever and small pox should be catered.

Treatment

Earmarked hospitals should have specialized CBRN treatment centres with trained specialist and paramedics for management of CBRN casualties. Some of the advance care and diagnostics facilities for CBRN casualty management are as follows (2):

- Radiation injury treatment center.
- Advanced blood bank facility. Some of the important facilities include, blood component separation, apharesis, stem cell harvesting, immuno haematological, Infection markers screening, leucodepletion and gamma irradiation. The cold chain system also needs to be created such as an adequate storage unit including deep freezer (-33 deg C): ultra deep freezer (-83 deg C) platelet agitator cum-incubator (+22 deg C) and adequate blood transportation boxes.
- Advanced laboratory facility. This facility should have a genetic and

molecular laboratory and other specialized diagnostic facilities for CBRN management.

- Burn Centre
- Selected hospitals should develop bone narrow facilities.

Chemical Warfare

Chemical weapons are a potent means of mass destruction. The chemical agents used may be lethal agents like Sarin which generally kills the targeted group or incapacitating agents such as Distilled Mustard which temporarily incapacitate the victims.

Historical Facts

Blister agents were used by the German against the British during the First World War. There were almost 1,68,000 casualties due to this, with a death rate of two to three percent. Phosgene accounted for eighty five percent of deaths attributable to chemical weapons during the First World War. Mycotoxins are alleged to have been used in military warfare in Laos (1975-1983), Cambodia (1978-1983) and Afghanistan (1979-1983). They are the causative agents which have been allegedly responsive for the mysterious "yellow rain" phenomenon in these places.

The agents used in chemical warfare may be grouped as follows:

Nerve Agents

These interfere with the functioning of the nervous system and thus adversely affect human body functions such as respiratory and muscular activities. The main action of nerve agents is through inhibition of the enzyme cholinesterase. Acetylcholine is thus not neutralized and hence, continuously acts on receptors in the smooth muscles. Examples of this group are Tabun (GA), Sarin (GB) Soman (GD) and Vx. Their main route of entry in the human body is through the respiratory system and the skin. The principles of treatment include termination of exposure to toxic substance, establish/ maintain ventilation and specific antidotal therapy such as atropine (acts by blocking the effects of excess acetylcholine at muscarinic receptors) and oximes which reactivate the organophosphate inhibited phosphorylated enzyme. The standard antidote kit contains injection Atropine 2 mg and Injection 600 mg PAM-C.

Blister Agents

These are so called since they cause blistering of the skin. Examples of this group are Distilled Mustard (HD), Nitrogen Mustard (HN) and Lewisite (L). They affect by interfering with DNA

synthesis and cellular division. They are cytotoxic as well as mutagenic. They gain entry in the human body through the oral, respiratory system and the skin. These agents were successfully used by the Germans against the British during the First World War. The antidote used for Lewisite poisoning is BAL (Dimercaprol).

Blood Agents

These agents are absorbed by inhalation and prevent body tissues from utilizing the oxygen in the blood e.g., Hydrogen Cyanide (AC), Cynogen Chloride (CK) and Arsine (SA). These agents combine with cytochrome oxidase enzyme which is essential for oxidative process of the tissues and hence oxygenation of the tissues is affected. Amyl nitrite and sodium nitrite with or without sodium thiosulfate are used as antidotes for cyanide.

Choking Agents

These affect the respiratory system, e.g. Diphosgene (DP), Chloropicrin (PS) and Phosgene (CG). The result is pulmonary edema, hypoxia and haem concentration. Phosgene was used as a major chemical warfare agent during the First World War.

Tear Agents

These are often used as riot control agents.

Incapacitating Agents

These include substances such as BZ and LSD. They produce physiological or mental effects or both rendering individuals incapable of performing their assigned duties. The signs and symptoms include those associated with anti cholinergics, indoles and cannabinoids.

Biological Warfare / Bio Terrorism

Biological warfare / bioterrorism is the use of living organisms or their toxic products to cause death, disability or damage to man. Though the biological weapons have seldom been employed, there is ample evidence that there are nations and terrorist groups that have the capability and already have stocks of these weapons. The biological agents may be lethal and transmissible, lethal and non transmissible, incapacitating and transmissible and incapacitating and non transmissible. The review of the events related to bioterrorism reveals the following (3):

- Bioterrorism is more likely in use than ever before and far more to be feared than either explosives or chemicals.
- Civil population has scarcely been targeted and the subject of bioterrorism is hardly discussed publicly.

- Recipes for making biological weapons are now available on the internet.
- Detection or interdiction of those intending to use biological weapons is extremely difficult.

Historical background

Biological warfare has been used since long. In the 14th Century, the targets besieging the Italians in a fortress in Cremea threw over the wall the bodies of plague victims forcing abandonment of the fortress. The Italians also published a tactical manual describing how to construct artillery shells for delivery of disease carrying organisms. It has also been documented that the Red Indians in North America were surreptitiously given small pox infected blankets. Limited biological warfare is reported to have been carried out by Japan during World War II. In the recent past, mycotoxins have been reported to be used in Afghanistan.

In 1972, Biological and Toxic Weapons Convention attended by almost all the countries agreed to cease bio-weapons research programs and to destroy stocks of bio-weapons which they possessed. Inspite of this, it has been reported that even in 1990s, countries had stockpiled tons of dried spores of anthrax, smallpox, plague and tularemia.

Biological Agent Dissemination and Delivery Techniques

The main methods for disseminating the agents are through aerosols or by the use of disease carrying vectors (4). Explosive bomblets in which there is burster, surrounded by biological agents and enclosed in thin case, explode upon impact and disseminate the biological agent as an aerosol. Spray tanks carried by aircraft and missiles may also be utilized for producing aerosol containing the biological agent. Disease carrying vectors such as mosquitoes, mites, tics and lice may be delivered by aircraft or missiles in containers which rupture on impact. The biological agents may also be introduced into the food chain or water.

Biological Warfare Agents

Various micro-organisms have been studied as biological warfare agents such as the Dengue virus, Ebola virus, Lassa virus, Haemorrhagic Fever viruses, Rickettsea Prowazeki, Coxiella Burneitte, Bacillus Anthracic, Vibrio Cholerae, Yersinea Pestia, F. Tularensis, Salmanele Typhae and so on. The agents most likely to be used are spores of smallpox, anthrax and plague. The bacterial toxins that are utilized are Botulin, Staphylococal, and Tetanus whereas the mycotoxins include alphatoxins and trichottoceus.

Diagnosis

Biological agents can multiply only in a living host and there is no definite sensor available to detect the agents. An early accurate diagnosis is the key to manage casualties of biological warfare. Samples from patient suspected to have been affected by biological warfare agent must be sent to designated medical facilities. The confirmatory laboratory diagnosis may be done by antigen, antibody detection. By application of fluorescent antibody technique (FAT), the identification of biological warfare agents such as virus causing smallpox may be done in one to two hours.

Epidemiological Clues of a Biological Warfare or Terrorist Attack

These include the following

- The presence of a large epidemic of a disease, in a discrete population.
- Many cases of unexplained diseases or deaths.
- Disease more severe than is usually expected for a pathogen or failure to respond to standard therapy.
- Unusual routes of exposure for a pathogen, such as the inhalation route for disease that normally occur through faeco-oral route.
- A disease that is unusual for a given geographic area or transmission season.
- Disease normally transmitted by a vector that is not present in the locals area.
- Multiple simultaneous or serial epidemics of different diseases in the same population.
- A single case of disease by an uncommon agent. (Small pox, some viral hemorrhagic fevers).
- A disease that is unusual for an age group.
- Unusual strains or variants of organisms or antimicrobial resistance patterns different from those circulating.
- Similar genetic type among agents isolated from distinct sources at different time or locations.
- Higher attack rates in those exposed in certain areas, such as inside a building if released indoors, or lower rates in those inside a sealed building if released outside.
- Disease outbreaks of the same illness occurring in non contiguous areas.
- A disease outbreak with zoonotic impact.

- Intelligence of a potential attack claims by terrorist or aggressor of a release, and discovery of munitions or tampering.

Detection

Accurate intelligence is required to develop an effective defense against biological warfare. Once an agent has been dispersed, detection of the biological aerosol prior to its arrival over the target, in time for personnel to don protective equipment, is the best way to minimize or prevent casualties. However, interim systems of detecting biological agents are just now being fielded in limited numbers. Until reliable detectors are available in sufficient numbers, usually the first indication of a biological attack in unprotected soldiers will be the ill soldier.

Detector systems are evolving and represent an area of intense interest with the highest priorities within the research and development community. Several systems are now being fielded. The biological integrated detection system (BIDS) is vehicle mounted and concentrates aerosol particles from environmental air then subjects the particle sample to both genetic and antibody based detection schemes for selected agents. The long range biological standoff detection system (LRBSDS) will provide a first time biological stand off detection capability to provide early warning. It will employ infrared laser to detect aerosol clouds at standoff distance up to 30 km. An improved version is in development stage. This system will be available for fixed site applications or inserted into various transport platforms such as fixed wing or rotary aircraft and short range biological standoff Detection System (SRBSDS) is in the research and development phase. It will employ ultraviolet and laser induced fluorescence to detect biological aerosol clouds at distance effective range 5 km. The information will be used to provide early warning, enhance contamination avoidance efforts and cue other detection efforts.

The principal difficulty in detecting biological agent aerosols stems from differentiating the artificially generated BW cloud from the background of organic matter normally present in the atmosphere. Therefore, the aforementioned detection methods must be used in conjunction with intelligence; physical protection, and medical protection (vaccines and other chemo prophylactic measures) to provide layered primary defenses against a biological attack.

Networking of laboratories under Integrated Disease Surveillance Program (IDSP)

Under the IDSP of Government of India, a laboratory net work has been established at various levels of health care as given below and this network is to be used for disease surveillance/ outbreak investigation even during disaster. The network comprises of Peripheral Laboratories and Microscopy Centers (L1 labs); District Public Health Laboratories (L2 Labs); State Laboratories (L3Labs); Regional and Quality Assurance Laboratories (L4); Disease based reference laboratories (L5). There are 8-10 laboratories of repute in the country which will be acting as National Reference Labs. The IDSP will support the biological disaster management also, in order to set up the district laboratories initially there will be strengthening of 3-4 priority laboratories for each state and model district labs will be set up which will later on be expanded to all the district laboratories.

Personal Protection

If outdoors, personnel should keep the head covered, wear a scarf and cover the nose with a handkerchief/cloth. The currently fielded chemical protective equipment, which includes the protective mask, battle dress over garment (BDO), protective gloves, and over boots will provide protection against a biological agent attack. At the earliest, refuge should be taken in a closed shelter. Ultra high efficient filter masks which are capable of filtering more than 99 percent of particles of 1-5 microns should be utilized.

Collective Protection

All doors and windows of buildings must be closed when a biological attack is imminent. For effective protection persons should take shelter in a building with an efficient air filtration system.

Decontamination

Decontamination involves either disinfection or sterilization to reduce microorganisms to an acceptable level on contaminated articles. BW agents can be decontaminated by mechanical, chemical and physical methods:

- Mechanical decontamination involves measures to remove but not necessarily neutralize an agent. In a BW context, the use of an air filter to remove aerosolized anthrax spores, or water to wash agent from the skin.
- Chemical decontamination renders BW agents harmless by the use of disinfectants that are usually in the form of a liquid, gas or aerosol.

- Physical means (heat, radiation) are other methods than can be employed for decontamination of objects.

Immunoprophylaxis

Vaccines against a number of potential biological warfare agents have been developed; anthrax, small pox and some including polyvalent vaccines are in various stages of research. Troops of the multinational force in the Gulf War were reported to have been protected against Anthrax.

Chemoprophylaxis

Chemoprophylaxis would be useful if the biological warfare agents have been identified, such as Anthrax, Plague, Q Fever, Glanders and Melioidosis.

General Measures of Protection

The general population should be educated and the concerned must understand that biological warfare/bio-terrorism is a possibility.

- Only cooked food should be consumed.
- Boiled / chlorinated / filtered water should be consumed.
- Insect and rodent control measures must be initiated at the earliest.
- Isolation of suspected / confirmed cases preferably in negative pressure isolation.

Major Components of CBRN Management (2)

CBRN Emergency Van

Hospitals with CBRN casualty treatment centres must have a CBRN emergency van equipped with CBRN detection, protection and decontamination equipment and material.

Radiation Detection

The following detection equipment is essential for rescue teams and QRMTs (Quick Reaction Medical Teams)

- Personal Radiation Dosimeter: It gives a direct visual reading and a safe radiation range can also be set on the equipment.
- Thermo Luminescence Dosimeter (TLD) Badges: The TLD gives the information about the cumulative radiation does.
- Radiation Dose Survey Meter: It can be hooked with a computer and data can also be transferred through telephone lines.
- Micro Bomb Detectors: It contains a real time alerting mechanism through a bright light and buzzer.
- Vehicle Detectors: A moving vehicle monitor designed for measuring and determining radioactivity of vehicles should be provided at all entry and exit gates.

- Whole Body Counters: Whole body counters will detect any radioactive material inside and outside the body.

Chemical Detectors

Chemical agents monitors, AP2C, 3 colour detector papers, portable gas chromatographs, residual vapour detection kits.

Biological Integrated Detection System

It is a high mobility, multi-purpose, wheeled vehicle mounted system that concentrates aerosol particles from air, then subjects the particle sample to antibody-based detection schemes for selected agents.

Protective Equipment

Protective equipment is required by rescue teams and QRMTs, for evacuation of victims from the contaminated area.

Creation of CRBN Decontamination Room

Earmarked hospitals must have a decontamination room having appropriate equipment and material. The decontamination room should have a lightweight durable, impermeable, washable and reusable fiberglass tabletop with a flexible drain hose, locking straps spray nozzle and wall mounting bracket. Two 100-litre waste collection containers must also be available. All nuclear casualties should be decontaminated prior to shifting into the treatment ward.

CBRN dust filter fitted ward

CBRN casualty treatment ward must be fitted with CBRN filtration units to provide purified air with positive pressure inside so that contaminated air cannot come in.

Special Laboratories

- Radio Bio-dosimetry Laboratories – Radio Bio-dosimetery includes lymphocyte estimation along with the other formed elements of the blood. Chromosomal study is an important tool for radiation bio-dosimetry (3).
- Hematology Laboratories/Blood Banks. Blood and bone marrow are very sensitive to radiation. Following radiation exposures, neutropaenia will occur suppressing the immunity of the casualty leading to infection. Therefore a hematology laboratory/ blood bank with a cell separator for granulocyte concentrates is an essential requirement for the management of radiation injuries.
  (a) Genetic Laboratories. Genetic studies must be carried out in a properly equipped genetic

laboratory for proper monitoring surveillance and counseling of victims.

(b) Molecular Laboratories. Radiation injuries damage DNA, therefore a molecular laboratory needs to be established in the radiation injury treatment centre for DNA and other molecular studies.

(c) Immunology Laboratories. The immunology laboratory will facilitate studies in cell mediated and humeral immunity. The laboratory will also be useful for antibody detection of various biological agents.

Bone Marrow Bank. For the restitution of the immune system, bone marrow transfusion is very important. The bone marrow of a person showing high risk of radiation exposure will be harvested, cryo-preserved and stored to transfuse at the time of requirement. Therefore, stem cell harvesting facilities and bone marrow banks are essential components of acute radiation injury treatment centres.

Immunoprophylaxis and Chemoprophylaxis. Prophylactic immunization is an important means for providing protection against biological agents. For some biological agents the only available counter measures might be specific anti-serums. Chemoprophylaxis using broad spectrum antibiotics offers an additional option for biological agents. Some cases like anthrax may require coupling of antibiotics with vaccines.

CBRN Management Equipment for QRMTs (2)

These include the following

- CBRN Detectors
- CBRN Protection and Decontamination Equipment
- Material for Area and Equipment Decontamination
- Decorporation Drugs
- Chemical Casualty Treatment Kit

Conclusion

NBC warfare as a full fledged attack or as a limited tactical strategy by countries or terrorist groups is a possibility. The casualties will include the army personnel and the civilians. The magnitude of casualties and the complex nature of the injuries sustained as a result of NBC attack require a comprehensive management planning. Dissemination of information and training of all concerned i.e. army, general public, fire brigade, police and other organizations must be carried out

before a NBC disaster occurs. It is essential that a comprehensive planning is done for protection and management of NBC casualties. Networking including integration in the existing medical and administrative set up must be planned and executed. Understanding, planning and implementation of NBC casualty medical management set is a national challenge for the present. The future of mankind may depend on its effectivity.

References

1. Michael J (1980). Medical Aspects of Nuclear Warfare: A Review. *Military Medicine* **145(4):** 243-45.
2. National Disaster Management Authority, Govt. of India. *National Disaster Management Guidelines – Medical Preparedness and Mass Casualty Management Oct 2007.*
3. Danzig R, Berkowsky PB (1997). Why should we be concerned about Biological Warfare ? *JAMA* **285**:431-32.
4. Vorobyov A (1994). Criterion Rating as a Measure Of Probable Use Of Bio Agents As Biological Weapons. Paper presented at a meeting of the Working Group on Biological Weapons Control of the Committee on International Security and Arms Control, National Academy of Sciences.

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Stem Cell Therapy In Stroke

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Abstract

The ultimate aim of any therapeutic strategy is the maximum functional restoration possible and eventual complete normalcy of function. The non – regenerative capability of the injured adult brain has been challenged in recent years and neural plasticity has been observed experimentally in both global and focal brain ischemia in animal models. Whether neuro - genesis increases in response to brain lesions and whether stem cells can be used for transplantation are potential questions to be answered. Functional recovery may occur in a small or localized brain injury using rehabilitation measures, but for large ischaemic strokes, the restoration may require new synaptic connections within and away from the damaged tissue. In an infarcted area, the ischemic core may not respond to any pharmacological or rehabilitative intervention. For these reasons, the prospects of repairing the neuron system, using cell transplantation, seems promising and may offer a unique approach for brain repair and restoration of function. On-going animal and human trials have largely helped in burgeoning our hopes on this method of restorative therapy after stroke.

Key words: stem cells, stroke, neuroregeneration, brain plasticity, restorative therapies

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Introduction:

"Physicians have always inspired two powerful sentiments in the contemporary society: the hope that they will create a healthier world than their forefathers without discarding fundamental values of professionalism and ethics, and the fear that they will make things worse by following pseudoscience which is misleading, wasteful, lacking in vigilant peer review, and advocated by drug and devices industry." These are the opening words by Prof. Bajaj in his preface of the Annual Medicine Update this year (1). There is no better way to aptly describe the hope and hype regarding stem cell therapy. The ultimate aim of any therapeutic strategy is the maximum restoration possible and eventual complete normalcy of function. The nervous system has little capacity for self repair. However, the non – regenerative capability of the injured adult brain has been challenged in recent years and neural plasticity has been observed experimentally in both global and focal brain ischemia in animal models. Neuroimaging studies in stroke patients indicate altered post stroke patterns suggesting functional reorganization. However, whether neuro - genesis increases in response to brain lesions and whether same stem cells or progenitor cells present in brain be used for transplantation are potential questions that need to be answered. Recent studies have shown in- vivo differentiation of progenitor cells into neurons in adult human dentate gyrus. Functional recovery may occur in a small or localized brain injury using rehabilitation measures, but for large ischaemic strokes, the restoration may require new synaptic connections within and away from the damaged tissue. Considering the relatively poor capabilities of neural self-regeneration, this seems quite impossible. In an infarcted area, the ischemic core may not respond to any pharmacological or rehabilitative intervention. For these reasons, the prospects of repairing the neuron system, using cell transplantation seem promising and may offer a unique approach for brain repair and restoration of function. Considering the fact that the neuronal circuitry is a complex array of neurons and connections and the prospects of this technique at first thought seem remote, yet, the growing evidence from animal models and small clinical trials has suggested the possibility of reconstruction of neuronal network, making the aspect of restorative medicine significantly promising.

Compared with neurodegenerative disease, stroke poses special conditions that impact the potential success of

transplantation to enhance neurological recovery, including the anatomy and time of stroke, the vascular supply, site of implantation, and type of patients enrolled in clinical trials. In contrast to a neurodegenerative disorder such as Parkinson's disease (PD), which destroys a relatively homogenous population of neurons, strokes affect multiple different neuronal phenotypes. An infarct might involve the thalamus, hippocampus, and striate visual cortex affecting 3 or more very different neuronal populations. Besides, oligodendrocytes, astrocytes and endothelial cells are also affected. Reconstitution of the complex and widespread neuronal-glial-endothelial interrelationships may require cells for transplant to initially remain immature and phenotypically plastic to differentiate into appropriate neural, glial and endothelial cell types depending on the ectopic site. If white matter is destroyed in a stroke, cell implants may not produce functional connections with axons that can penetrate through the scar tissue of a chronic infarct.

There is uncertainty about the mechanism(s) by which cell transplantation might improve stroke deficits. Transplanted cells would ideally replace cells that are damaged by ischemia and take over function of these cellular elements. However, it is also possible that transplanted cells secrete trophic factors that help to maintain marginally surviving cells or otherwise enhance the local environment sufficiently to improve function. Transplantation might also conceivably produce a host reaction that could include sprouting of new axons and synapse formation.

In the understanding of the efficacy of different treatment strategies on human brain, appropriate animal models are imperative which mimic human disease. Two major types of ischaemic models are studied: a) global and b) regional or focal. The global ischaemic model (produced by ligating vertebral and carotid arteries for 5 – 15 min) mimics the effect of cardiac arrest or coronary occlusion in humans and focal models replicate the consequences of stroke. The effects of focal ischaemia are seen on discrete regional group of neurons, especially CA1 neurons of hippocampus and neural transplantation into this region has been studied with benefit in rat ischaemic models. Improvement in this model however requires replacement of dead CA1 neurons and graft – host connectivity. The focal ischaemia models are produced by MCA ligation techniques thereby producing focal brain infarctions, generally in region of

striatum or cortex. Fetal cortical grafts placed in infarcted regions have shown to develop afferent connections from cortex, thalamus, and sub cortical nuclei of the host, whereas efferent connections are sparse, seen to improve with the housing of animal in an enriched environment.

Cell types and sources of intracerebral grafting (2-6)

The most important issue in any cell transplantation technique would be the availability of appropriate cell type having the ability to proliferate *in vivo*. The therapeutic effects of implanted neurons or neuronal precursors are likely to be successful if they structurally and functionally integrate into the brain. It is important that cellular elements used in transplantation are immature i.e., at a developmental stage where they are terminally differentiated but have not formed connections and are phenotypically plastic so as to differentiate into different cell types i.e., neural or glial depending upon the site of implant. Although, the ability to develop neuronal connectivity is maximum during the fetal period, nevertheless, the adult brain has the capacity to establish functionally active connections, especially during the periods of injury thereby suggesting reactivation of regulatory mechanisms during degeneration or insult. Following cell types have been studied as potential candidates for neural repair in ischaemic stroke.

a) Embryonic/ Fetal cells (7-14)

Fetal tissue has been the major source of cells for transplantation in animal models of stroke. As mentioned above, the likelihood of survival is best for immature cells, before they have arborized their axonal network. Transplanted fetal cells have shown to survive, integrate and improve deficits in animal models of neurodegenerative disease, as well as PD in humans. The gestation age of 14 – 20 days is generally used in animal models. For fetal hippocampal and cortical donor cells, days 18 – 20 and for fetal striated cells, less than 16 days have been generally used in majority of studies. Since there are major ethical and legal issues governing the use of "human fetal embryonic tissue", other cell sources are being seriously considered and investigated, as studies have shown that multiple donor or first trimester tissues are not perquisites to generate long term surviving human brain grafts. Owing to the short supply of human donor material and ethical concerns about the human embryos as donors, animal tissues have been considered as an alternative source with advantage of being genetically modifiable. Pigs are

useful as donors as they are non – endangered species and produce large litters as opposed to non – human primates. Transplantation of fetal cells from primordial striatum of porcine origin, known as lateral ganglionic eminence (LGE) was shown to improve function in rat ischaemic models. In small scale trials, porcine tissue has been used to treat patients with PD, HD, focal epilepsy and stroke with partial success. However, some issues regarding use of this cell source necessitate pondering. Studies have shown that the cells tend to retain original phenotype of striated origin even if implanted into non – striatal regions making use of these cells into stroke regions outside striatum questionable. The likelihood of graft rejection in humans is of potential concern and strategies need to be devised to overcome this. The risk of host contamination of viruses is of immense concern. It has been reported that porcine endogenous retrovirus particles (PERV) could be released from the porcine cell lines and can infect human cell lines. Since then a debate on PERV infection from xenotransplantation or its integration into human retrovirus, with resultant novel mutations has been ongoing. Guidelines call for regular monitoring of patients undergoing xeno-transplantation.

b) Immortalised cell lines (15,16)

In view of the ethical difficulties in transplanting embryonic cells and technical problems in xenotransplantation, alternative sources of graft cells have been devised. One of these cell lines, called "immortalized cell lines" have been an important technical advance in the field of neurotransplantation. These cell lines are derived by infecting neuroepithelial precursor cells from predefined CNS regions before their terminal mitosis, with a retrovirus encoding an immortalizing oncogene. Individual clones are then isolated and characterized e.g., MHP36, MK31, Ht9–C7 etc. The tissue sources have been variable i.e., mouse hippocampus, hypothalamus, mesencephalon, mouse striatum and others. The transforming protein is generally expressed, even if the cell is being pharmacologically induced to differentiate, making it of potential concern both *in vitro* and *in vivo*. Data from studies suggest that the neural precursor cell lines are plastic, and have ability to differentiate into multiple lineages *in vitro* and can respond to local microenvironmental cues. However, these cell lines do not behave similarly either *in vitro* or *in vivo*. Probably, each cell line responds to a set of effector molecules *in vivo*. The advantage of establishing an

immortalized cell line is in providing an unlimited number of identical cells from a single cell propagated in culture, higher level of neurotransmitter production using genetic manipulation, better pooling and sorting of viable cells, screening for infectious diseases and efficient planning of surgical procedure.

c) Spontaneously arising neural cell lines or neuron like cells (17-20)

Neuroblastomas and glioblastomas are the chief spontaneously arising neural cell lines. These contain cells of mixed population which are often undefined. Embryonal carcinoma (EC) cells are derived from spontaneously occurring testicular germ cell tumors and can differentiate into both neural and non – neural cells. In response to therapy with retinoic acid, the mouse derived EC cell line (P19), differentiates into neurons, astrocytes and oligodendrocytes. However, neural transplantation studies on rat striatum showed that these cells tend to retain their original characteristics established *in vitro* and have phenotypic plasticity *in vivo*. Although tumorigenicity has not been observed, the risk is potential once transplanted.

N–Tera–2 Cells were derived from human testicular germ cell tumor, years ago. Also called LBS – neurons (after Layton Bioscience Inc. Ath. Cal), the credit of development and patenting of the process to cleverly transform this rapidly dividing cell line into fully differentiated non – dividing neurons goes to researchers at University of Pittsburgh, Pennsylvania. Upon several weeks treatment with retinoic acid (an agent known to produce maturation of cancer cells into their normal looking non – cancerous equivalents) and mitotic inhibitors, an enriched population of post – mitotic differentiated neurons known as NT2N or HNI cells, showing an exclusive commitment to neural lineage were produced. They have been seen to closely resemble neural precursor cells, and express cell surface markers and cytoskeletal proteins unique to neural stem cells. They represent a well characterized and unlimited source of human neurons for transplantation that can be reproducibly generated. NT2N cells, are "Neuron Like Cells" as they have a symmetrical morphology, elaborate an extended axon and elongated dendrite. These cells can express neurotransmitters, functional glutamate receptors, calcium channels and proteins capable of secretory activity and synaptogenesis. The ready constant availability of cryopreserved pure neurons has made the NT2N cells an attractive graft source and trials in

animal studies and initial results in ongoing clinical trials in humans are encouraging. The use of these neurons obviates the need for fetal cells, which has raised ethical concerns especially regarding elective abortions. Spontaneous abortions are rare and unpredictable events, so harvesting tissues from these fetuses would be impractical. Further, cells from spontaneously aborting fetuses are likely to harbor genetic defects. Another feature of the LBS – Neurons is that they can be frozen and transported to clinical centre for transplantation and thawed without damage before use.

d) Stem cells (21-23)

The discovery of adult tissue specific stem cells, such as hematopoetic stem cells, having ability to differentiate into other tissues has generated immense interest among cell biologists and transplant clinicians. Stem cells are cells capable of proliferation, self maintenance and production of differentiated functional progeny that are characteristic of the organ from which they are derived. In adult animals stem cells are present in organs like bone marrow, skeletal muscle, intestine, liver, peripheral nervous system and retina etc.

The long stranding dogma of adult mammalian brain lacking neurogenesis and evidence of progenitor cells, has been recently challenged by studies showing continuous neurogenesis in olfactory bulb, hippocampus and dentate gyrus, from the neural stem cells (NSCs). These NSCs are defined as undifferentiated cells that are able to self – review as well as generate the three major cell types that constitute the CNS: neurons, astrocytes and oligodendrocytes, signifying their pluripotent nature.

These features have lead to many studies aimed at characterizing, isolating, expanding and transplanting these fascinating cells. Whether neural stem cells meet all these criteria is still unresolved. It is likely that cell lineages generated from NSCs differ among stages and regions of the CNS, e.g. in the cortex there may be selective progenitor cells giving rise to neurons (neural progenitor cell) or astrocytes and oligodendrocytes (glial progenitor cell). The NSCs can be expanded to a particular clone using free floating "neurosphere" cultures and the lineage potential can be assayed using clonal monolayer cultures. The identification process and isolation of NSCs is tedious and difficult. There are selective marker molecules for NSCs e.g., mushashil (RNA binding protein), nestin (intermediate filament) and the members of SOX family, but are not cell

surface antigens unlike haematopoetic cells.

What is the localization of neural precursor or stem cells? In the embryonic stage, they have long been believed to be located in the ventricular zone of the neural tube after neurulation and this is consistent with immuno chemical staining analysis using mushashil. In the adult rodents, neurogenesis is seen to occur in dentate gyrus and olfactory bulb and has been experimentally shown even in adult humans. The sub ventricular zone (SVZ) and ependymal layer also corresponds to neurogenesis site in adult brain. Regeneration may also be made effective by stimulating the endogenous neural precursor cells or stem cells by injury, as has been shown in studies, either spontaneously or by using exogenous stimuli like neurotrophic factors (BDNF) administered intraventriculary or by infusion. Thus, it is likely that adult brain parenchyma may recruit and/ or generate new neurons, which could replace the lost neurons.

e) Bone Marrow Stromal Cells (24-28)

Over the years, tissue specific stem cells have been shown to give rise to cells, not normally found in the organ or tissue of residence. The plasticity of adult stem cells opens up possibility of using autogenous adult stem cells to treat various disorders (38). The bone marrow stromal cells (BMSCs) provide structural and functional support for the generation of blood cell lineages from haematopoeitic stem cells e.g., fibroblast reticular cells, adipocytes, macrophages etc, and under specific conditions differentiates into a variety of tissue, e.g. bone, cartilage, muscle, glia and neurons. When exposed to epidermal growth factor or neurotrophic factors like BDNF *in vitro*, or cultured with neural cells, human BMSCs differentiate into cells, expressing neural precursor cells (NPC) markers. Sanchez – Ramos *et al* used retinoic acid (RA) in combination with growth factors to induce differentiation of BMSC to neural phenotypes whereas others have used â-mercaptoethanol for inducing rapid differentiation into neuron-like cells. The advantages with these cell lines seem many. Obtaining marrow cells would be easy and expanding them in culture would not be that difficult. Using patient's own BMSC would theoretically eliminate the risk of rejection. However, differentiation mechanism for these cells is poorly understood. Whether these cells truly produce neuronal synaptic network with plasticity or produce trophic factors alone is questionable and speculative. Issues like long-term

survival, safety, plasticity and behavior of BMSCs need further evaluation before clinical use.

f) Umbilical cord blood cells

Human umbilical cord blood may also harbor cells (human umbilical cord blood cells HUCBs]) capable of differentiation into neural lineages. When exposed to nerve growth factor and RA, the derived umbilical cells produce progeny that show positivity of neural and glial cell markers. However, biology of the cells is currently poorly understood, and it is likely that positive effects of these cells are related to their neurotrophic action, rather than actual neuronal circuitry formation. A better understanding of these cells is needed before clinical transplantation studies are undertaken, although experimental data in animal models of stroke have shown functional benefits.

How do transplanted cells work? (24, 29)

In most cases of neural transplantation, it is likely that therapeutic effects of the implanted neurons or their precursors would be dependent upon their functional and structural integration into the brain tissue. However, the question is whether establishment of neural circuitry the only means of improvement. It is likely that transplanted cells release neurotransmitters or neurotrophic/ neuroprotective factors which counteract degeneration or promote regeneration. Even transplanted glial cells have been used to modify response to injury and assist in structural repair and promote remyelination. Studies using bone marrow stromal cells or umbilical cord blood cells as potential donors have shown functional improvement in behavioral recovery in animal models within days of transplantation. This raises issues whether recovery observed in such short periods is related to release of trophic factors rather than engraftment and differentiation of transplanted cells into mature neurons and / or glia. The functional benefits after neural transplantation are likely to be mediated by one of the following mechanisms.

1. Neurotransmitters released from the graft tissue act on the afferent deprived limb of the post synaptic receptors.
2. Release of the neurotrophic / growth factors (brain derived neurotrophic factor [BDNF], glial derived neurotrophic factor [GDNF], nerve growth factor [NGF] etc) acting as local pumps to support cell function and to prevent cascade of apoptosis. Regenerating neuronal population

further prevents subsequent cell death.

3. Reestablishment of local interneuronal connections and synaptic connectivity between the host and graft.
4. Cell differentiation and integration.
5. Improvement of regional oxygen tension.
6. Limit glial reaction and prevent retrograde degeneration.

Possibly, the overall success of functional outcome is mediated by a combination of the above mentioned factors. Taking the model of PD, it is easy to understand the mechanisms operating for improvement as a specific cell function restoration is needed along the dopaminergic neurons. Ischaemic stroke, however, damages several neuronal circuits and not specific cholinergic or dopaminergic pathways, and therefore, neural transplantation is likely to be less effective. Restoring circuits, and enhancing cell survival can be achieved by cell transplantation. However, improvement might require reconstruction by long distance projection systems requiring multiple site implantation, and "bridging grafts" as explored in PD, may be applied for ischaemic lesions in future for bridging intact host tissue with ischaemic penumbra and core.

Cellular support for survival, integration and functional outcome

One of the most important factors deciding the fate of the graft is the trophic support offered by the intrinsic milieu, the host environment or administered *in vivo* postgrafting. Neurotrophic factors (NTFs) have been studied intensely as agents promising brain recovery in neurodegenerative disorders and acquired conditions like trauma or stroke. Neural differentiation of neuronal and glial cells and their maintenance are under the control of NTFs. In addition, an excessive administration of NTFs greatly protects sensitive brain tissue from injury. It is now thought that neural stem cells have the inherent potential to compensate and recover neural functions lost after ischaemic stroke as discussed previously. However, even the stem cells are under the control of NTFs to differentiate into a certain species of neural cells. In the present date, the number of growth factors available and likely to affect survival and development of progenitor cells is probably the largest. Astrocytes and endothelial cells surrounding or infiltrating the transplant are susceptible to the effects of platelet derived growth factor (PDGF) which is likely to mediate survival of graft through neovascularization. BDNF has

been closely related to graft survival of dopaminergic neurons or their precursors. It is now accepted that BDNF and its receptor trk B are widely distributed, both in developing and mature CNS. Currently, BDNF is most useful in cell transplantation to promote cell differentiation pre- implantation. It has been observed that under conditions like ischaemia and seizures, NTFs like BDNF, GDNF, NGF etc; are induced in brain cells. However, each NTF species shows a different effect on temporal and cellular differentiation. In ischaemic brain, BDNF, GDNF and vascular endothelial growth factor (VEGF) are seen to be upregulated within hours of stroke in experimental animal ischaemic models. Therefore, it possibly implies that neural transplantation may be made more successful with the concomitant use of these neurotrophic factors. Since development and plasticity of neural networks and connections are strongly influenced by the microenvironment of the host tissue, the conduciveness of this environment can be improved by either enhancing graft cell viability or improving host tissue responsiveness, achieved best by the use of trophic factors. In animal models of transplantation, housing conditions after the transplant affect outcome strongly. Whether the role of NFTs is confined to pre- implantation mechanism of differentiation of cells into mature precursors or later after transplanting in improving further differentiation, organization and structural integration into the host tissue is speculative. Although current data has not supported strongly their role in acute ischaemic stroke as neuroprotectants, their role remains strong and essential for neural transplantation studies. Thus transplant therapy, rehabilitative medicine and other strategies need to be combined together for a clinically effective and functionally active outcome.

Immunosuppression and transplantation

It is believed that host parenchymal interaction to the graft tissue is an important factor mediating success of transplantation. Clinical trials have explored the need for immuno suppression and contribution of graft vs host response during cell grafting. It is generally considered that brain is relatively immunologically benign and the concept whether immunosuppression is really necessary has been challenged. Herderson *et al* showed long term benefit of neural transplanting in PD patients in absence of immunosuppression. However, the above therapeutic benefits have been with the use of human fetal tissue, which theoretically may have less

immunogenicity mediating tissue acceptance. The concept however, may not hold true for the xeno-transplantation methodologies. Several immunological issues need to the solved before a clinical trial of xenogeneic neural transplantation. No concrete evidence of NK cell (natural killer cell) and complement activation mechanisms mediating neural xenograft rejection have been reported. Human serum contains not only contains anti-Gal, but also natural antibodies against other epitopes present in porcine embroyonic brain. Current immunosuppressive drugs are not efficient enough to protect neural xenografts from rejection, thereby necessitating development of alternative or supplementary strategies to reduce or abolish "T" cell response, a major element during xenograft rejection. Pretreatment strategies for donor host interactions might sound plausible but efficiency of pretreatment for reducing immunogenesis depends upon the donor-recipient combination used in evaluating them. For transplantation of porcine tissue in humans, optimal donor – tissue treatment is directed at both direct and indirect antigen arm of the T – cell response. Response of microglia towards the graft is one of the most intriguing aspects of host-graft reaction. Microglia act as antigen presenting cells, initiating immunological cascade, but also mediate survival of graft and host–brain regeneration with increasing evidence suggesting their beneficial role in CNS. In vivo, nigrostriatal dopaminegic neurons develop neurites around the wound or graft correlating with active neurotrophic factor production by macrophages and microglia. It is thus difficult to understand the behaviour of microglial cells. Whether specific factors or signals promote undue inflammatory response, experimental data does support their beneficial and neurotrophic effects on the CNS.

Types of stroke lesions amenable to stem cell therapy:

Not all stroke lesions may be amenable to cell transplantation. Most preclinical studies involve intrastriatal implantation. Studies of the middle cerebral artery implantation rodent model have shown that the striatum is the primary site of damage and many believe that the resulting deficits in memory, learning and motor behavior are directly associated with striatal injury. Cortical lesions also may be accessible to transplantation, but infarcts involving white matter are more problematic. A proliferation of transplanted cells in the cortex may not necessarily repair underlying axonal damage. There is even rationale for neural transplantation in patients with

pure white matter infarcts, which require an entirely different therapeutic strategy. The size and extent of infarction involving major arterial territories will play a significant role in patient selection. In patients with widespread damage, the number of cells potentially needed to restore function may be daunting.

Timing of stem cell transplantation after stroke:

The appropriate time to transplant after a stroke is unknown. In the acute setting, release of excitotoxic neurotransmitters, free radicals, proinflammatory mediators might threaten new tissue introduced into the peri-infarct region. Also, cells may be dying by apoptosis in the penumbra for several weeks after stroke. Inflammation leading to microglial activation may inhibit endogenous neurogenesis and may thereby suppress the growth and survival of transplanted cells.

On the other hand, in the acute stage, local repair processes are active, including the release of neurotrophic factors from the intrinsic milieu and the host environment during the early phase to facilitate implant growth, survival, differentiation and /or integration. The ischemic environment also promotes the generation of new neurons in periventricular regions and in the cerebral cortex. How transplantation will affect the on-going endogenous neurogenesis is unknown. There is accumulating evidence that stroke recovery involves plasticity of connections, which occur early after a stroke but may disappear months or years later. Transplantation might benefit from such plasticity and become maximally beneficial during this reorganization.

However, delaying the stem cell transplantation for several weeks after stroke must also contend with the disadvantage of formation of scar tissue which might adversely affect implanted cells. The choice of timing must also consider the natural course of recovery from stroke. Impairments have different courses of improvement depending on the type and severity. Many neurologists would therefore prefer to delay transplantation till the deficit plateaus. For these reasons and many others, some investigators have preferred to transplant at least a few months after a stroke. The two clinical trials have chosen to study disabled patients at least 6 months after a stroke. However, there are no corroborating animal models of chronic stroke. Few outcome measures exist for animals with chronic stroke infarcts. Most importantly, recovery in animals cannot

be easily equated across studies or related to humans.

Blood supply:

Transplantation is unlikely to succeed if there is a severe arterial occlusion without collateral circulation; inadequate blood supply would not support graft survival. In contrast, transplantation efforts in progressive degenerative disorders are not necessarily concerned with arterial patency and inflammation.

Site of Implant:

From a mechanical point of view, injection of cells into the fluid-filled cavity of a chronic infarct facilitates the migration of transplanted cells. Without a definable cavitated area, transplantation requires more direct pressure to inject risking damage to normal tissue. However, cavity fluid can dilute the concentration of donor cells.

In the acute setting, it may be appropriate to inject cells in the salvageable penumbra but grafts might still be exposed to the detrimental effects of spreading depression and excitatory neurotransmitters. Fetal cortical grafts to the ischemic brain have been shown to survive in the penumbra but not in the core lesion. However, in chronic infarcts, glial scarring might impede the delivery of cells to the penumbral areas.

Some investigators believe that grafts could be more effective if the poorly vascularized inflammatory environment of the ischemic region is avoided altogether and suggest the plausibility of transplantation to distant regions, even to the contra-lateral side.

Patient selection:

Patients selected for stem cell transplantation for stroke should have measurable deficits, impairments and handicaps. The neuroanatomical relationship between image-defined infarct and deficits should be well established. Co-morbidities and the need for extensive follow-up also play a strong role in determining which patients are good candidates for experimental therapies.

Cell types for stroke neuro-transplantation:

A range of different cell types under investigation for transplantation in experimental and clinical stroke trials are; NT2N, LGE, BMSC, HUCBC, NSC, and Adipose tissue cells. (Table-1).

Animal Studies : (30-51)

Stem/precursor cells from different sources have been tested for their ability to reconstruct the forebrain and improve function after transplantation in animals subjected to stroke. (Table-2)

Table-1 : Various Cell types under investigation for transplantation in experimental and clinical stroke trials

Cell type	Description	Advantages	Disadvantages
NT2N	Immortalized Cell line	Unlimited supply	Lineage restricted long-term safety
LGE	Fetal pig	Abundant supply	Lineage restricted Pig infections
BMSC	Bone marrow	autologous	Painful extraction IV approach may affect Other organs
HUCBC	Umbilical Cord blood cells	alternate source	Incomplete studies
Adipose	Stromal Stem cells	Abundant source	Incomplete studies
NSC	Neural Stem cells	Extraction from patient	Incomplete studies

Table-2 : Properties of Stem/Precursor cells grafted in animal models of stroke

Cell Source & transplantation approach	Effect on behavioral deficit
Rat SVZ precursors in rat cisterna magna	Improved sensorimotor function
Mouse neuroepithelial stem cell line in rat Cortex, striatum or ventricle	Improved sensorimotor function or spatial memory
Human fetal teratomcarcinoma cell line (NT2) in rat striatum	Improved passive avoidance task and symmetric motor behavior
Immortalized mouse cerebellar precursors On polymer scaffold in mouse cortex	?
Rat bone marrow stromal cells systemically Or in penumbra zone in rat striatum	Improved sensorimotor function and Neurological Severity Score
Human bone marrow stromal cells Systemically in rats	Improved sensorimotor function Neurological Severity Score
Human umbilical cord blood cells Systemically in rats	Improved sensorimotor function Neurological Severity Score

The transplants, including a mouse neuroepithelial stem cell line, the human NTera-2 cell line, and human bone marrow cells, have been reported to partly reverse some behavioral deficits. However, in most cases, the underlying mechanisms are unclear and there is little evidence for neuronal replacement. Only few grafted cells have survived and they have not exhibited the phenotype of the dead neurons. Moreover, it is unknown if the observed grafted cells are functional neurons and establish connections with host neurons.

Despite the poor evidence for significant neuronal replacement in these studies, improvement of various stroke-induced behavioral deficits has been observed. Stem cell transplantation probably can lead to clinically valuable improvements through several mechanisms. First, the tissue damage per se can stimulate plastic responses or interfere with neural activity in the host. Second, the transplants can act a biological minipumps and release a missing transmitter or secrete growth factors. These factors can stimulate plastic responses and improve the survival and function of host neurons. Third, the grafts can restore synaptic transmitter release by providing a local re-innervation. Fourth, and this is true neuronal replacement, the grafts can become integrated into existing neural and synaptic networks, and re-establish functional afferent and efferent connections.

Recent findings in rodents suggest an alternative approach to cell therapy in stroke based on self-repair. Stroke leads to increased generation of neurons from NSCs in the SVZ, lining the lateral ventricles. These immature neurons migrate into the damaged striatum, where they express markers of striatal medium spiny projection neurons. Thus, the new neurons seem to differentiate into the phenotype of most neurons destroyed by the ischemic lesion. However, because more than 80% of the new neurons die during the first weeks after stroke, they only replace a small fraction (about 0.2%) of the mature striatal neurons which have died.

Clinical Trials: (52-55)

NT2 Neuron cell trials:

Immortalized cell line NT2 are derived from a human testicular germ cell tumor more than 20 years ago. Unlike other teratocarcinoma cell lines, the NT2 cells show an exclusive commitment to a neural lineage when exposed to retinoic acid. Several studies have shown that NT2 cells resemble neural stem cells. They express cell surface markers and cytoskeletal

proteins unique to neural stem cells. Treatment with retinoic acid and mitotic inhibitors for several weeks ultimately results in the production of postmitotic, NT2N, which expresses neurotransmitters, functional neurofilament and cytoskeletal proteins, and other proteins indicative of secretary activity and synaptogenesis. Transplanted cells also release neurotransmitters and elaborate typical neuronal proteins.

Phase I:

Seven years ago, a clinical trial began to assess the safety of intrastriatal NT2N (produced by Layton Bioscience Inc. and known as LBS neurons for human use) transplantation in patients with basal ganglia infarcts and stable motor deficits 6 months to 6 years before transplantation. Twelve patients were treated with NT2N cell transplants and immunosuppressed using cyclosporine for 9 weeks. Based on preclinical safety data, doses of 2 and 6 million cells were considered appropriate. Four years after the study began, there have been on adverse events related to the implants. Two patients died of unrelated medical illnesses. On autopsy examination of one of these patients, who did not show clinical improvement and died of myocardial infarction, the graft site showed no signs of inflammation, neoplasia or infectious disease 27 months after implantation. Because NT2N cells are polyploidy for chromosome 21, grafted neurons were identified at the injection site with fluorescent in situ hybridization and DNA probes specific to this distinctive chromosomal feature. Positron emission tomography (PET) scanning at 6 months showed greater than 15% relative uptake of F-18 flourodeoxyglucose at the transplant site in six patients. This may reflect surviving and functioning implanted cells, enhanced host cell activity or an inflammatory response.

Phase 2:

A randomized open-label trial with observer blinded neurological evaluations was undertaken to test the effectiveness of neuronal cell transplantation in patients with substantial functional motor deficits following basal ganglia infarction. Fourteen patients were randomized to receive 5 or 10 million implanted cells followed by rehabilitation, compared with 4 patients who only underwent physiotherapy. Patients had stable motor deficits 1-6 years after the onset of stroke. Half the patients had an ischemic stroke, and the other half had a hemorrhage. The author tested the hypothesis that implantation of neuronal cells would be safe, feasible and improve motor neurologic deficits.

One patient had a single seizure and another had a subdural hematoma evacuated one month after transplantation. There were no cell-associated adverse events.

Functional outcomes were assessed by the National Institutes of Health Stroke Scale (NIHSS), European Stroke Scale Score, Stroke Impact Scale, Fugel-Meyer Score, and Action Research Arm testing. Cognition was also tested before treatment and after 6 months. Transplant patients showed a trend toward improvement in functional outcomes on several scales compared with baseline measurements before transplantation, but there were no statistically significant trends compared with the four controls. With such small numbers however, the significance of the findings is unclear. A third clinical trial will evaluate cell implantation for patients with stable cortical strokes.

Diacrin trial:

Phase I: A pilot safety and feasibility study was started in 1998. The original goal was to enroll 12 patients with chronic, stable, moderate-sized basal ganglia infarcts who would receive intrastriatal implantation of fetal cells from the porcine, primordial striatum, also called the LGE of porcine embryonic tissue and pretreated in culture with an anti-major histocompatability complex class I antibody, thus obviating the need for immunosuppression after transplantation. Five patients underwent transplantation. Their strokes occurred on an average five years earlier. Computed tomography at the completion of surgery showed no evidence of hemorrhage in any patient. The patients developed no new neurological deficits in the acute setting. One patient developed cortical vein occlusion thought to be related to the surgery, but the Food and Drug Administration terminated the study. At 2 years one of the patients showed improvement on the modified Rankin Scale.

The Korean University trial: (56)

This study was completed in 2005. It was a randomized controlled phase I/ II trial. Cell transplantation improved recovery from ischemic stroke in 30 patients with intravenous autologous mesenchymal stem cells infusion .They prospectively and randomly allocated 30 patients with cerebral infarcts with middle cerebral artery territory and with severe neurological deficits into two treatment groups : the MSC group (n=5) received intravenous infusion of 1x 10^8 autologous MSCs whereas the control group (n=25) did not receive MSCs. MSC treated patients received 5 x 10^7 cells twice : 4 to 5 (first boosting)

and 7 to 9 weeks (second boosting) over 15 – 20 minutes. Neurological deficits and improvements in function were compared between the groups for 1 year after symptom onset. Neuroimaging was performed serially in five patients from each group. Outcomes improved with the MSC treated patients compared with the control group.

Indian Scenario: Current Status

Pilot study conducted at All India Institute of Medical Sciences (AIIMS), in patients with acute ischemic stroke (randomized within one month of acute stroke) who have been injected intra-venously with autologous bone marrow derived mononuclear cells (BMMNCs), has proven safety of the procedure. The study was not designed for efficacy. Based on the results of the pilot study, a multicentric study co-ordinated by AIIMS and funded by Department of Biotechnology (DBT) is currently underway.

A department of science and technology (DST) funded study in patients with chronic hypoxic–ischemic injury to the brain including cerebral palsy with autologous intra-arterial injection of BMMNCs is also currently ongoing at AIIMS.

The National Brain Research Center (NBRC) at Manesar involved in research in neurosciences, is conducting basic work on understanding how the stem cells might work in neuronal plasticity and recovery.

Center for stem cell research has come up in CMC Vellore under the aegis of DBT and several others are in the pipeline for a bench to bed side research on stem cell treatments in various diseases.

There are reports of unmonitored, outside experimental protocol "treatments" with various varieties of "stem cells" for a variety of "incurable" diseases being given by "self-proclaimed" stem cell specialists in India and elsewhere reporting "un-paralleled" success stories in media. These reports have triggered an avalanche of patients and care givers of patients with degenerative disorders and as yet incurable diseases making a bee line to these unproven interventions by these unscrupulous practitioners who are making a fortune on public gullibility. Currently there seems to be no law to curtail these unscientific activities.

Adult Stem Cell Therapy in Stroke

Adult stem cell therapy for stroke can be divided in an endogenous and exogenous approach. The aim of the endogenous stem cell therapy is to exploit the population of adult stem cells already physiologically present either in

the CNS or hematopoetic system derived adult stem or precursor cells are administered locally or systemically after purification and propagation in culture.

Over the past decades, convincing evidence emerged that neurogenesis in the adult CNS is a continuous physiological process. Neurogenesis is present in two regions: the SVZ and the subgranular zone of the dentate gyrus. Additionally, recent studies also indicated the existence of NSCs in other regions of the CNS, namely the striatum, spinal cord and neocortex. External global stimulants such as enriched environment, physical activity and stress, or application of defined molecules such as fibroblast growth factor-2, VEGF, BDNF and erythropoietin differentially modulate adult neurogenesis and have been tried in experimental models of stroke.

Interestingly, acute cerebral ischemia in human subjects leads spontaneously to a threefold increase in CD34+ cell count in the peripheral blood. Considering this change as an insufficient self-repair mechanism, it is a logical consequence to further promote CD34+ cell mobilization pharmacologically by the administration of granulocyte colony stimulating factor (G-CSF). In addition, G-CSF has been described to exert neuroprotective effects following cerebral ischemia. A recent preclinical study found functional improvement in rats with focal G-CSF.

Both global and forebrain ischemia, or middle cerebral artery occlusion (MCA-O), have been shown to induce neurogenesis in the dentate gyrus in adult mammals. The main questions are whether newborns in these conditions are able to replace lost neurons at the lesion site in a specifically appropriate manner and whether neuronal replacement through endogenous neurogenesis can be correlated with functional recovery.

There are several concerns regarding stem cell therapy in stroke. First, what is the intended strategy for implantation? Transplanted cells may play a role as neurotrophin "pumps" rather than as grafted tissue restoring segmental connections. The mechanism of recovery might impact the choice of cells to implant. If a trophic response is desired, then a cell line genetically engineered to supply trophic support may be a better choice. If reconstitution of a neural network is desired, then access to a broader array of lineage species may be necessary to reconstruct the complex and widespread neuronal, glial and endothelial damage in stroke. It is crucial to better understand the basic biological mechanisms of individual cell types as implant sources for transplantation in stroke patients.

Such properties that need further clarification are proliferation, differentiation, potential lineage restrictions and extent of integration into host structures.

The major concern is safety. Long-term studies on the biological behavior of cell grafts are mandatory to better understand the effects and safety of cell transplantation in stroke. Just as transplanted cells in the heart could be arrhythmogenic, transplanted cells in the brain may prove to be epileptogenic. There remains virtually no information regarding the long-term effects on organ physiology and tumor formation from parenteral delivery of transplanted cells. The number of cells needed to promote recovery is a matter of debate. This issue impacts delivery methods given the volumes needed for adequate cell number. Lastly, the question regarding modulation of the transplanted tissue of the various responses after stroke, such as endogenous neurogenesis, synaptogenesis, angiogenesis and inflammation remains to be explored. Induction of endogenous neurogenesis also remains unexplored and is potentially very attractive.

In conclusion, basic and clinical research in stroke neurotransplantation remains in a nascent stage. Much more work is needed to further characterize the biology of different implant sources both *in vitro* and *in vivo*.

References

1. Bajaj JS (2008). Medicine : A Challenge and a Promise. In : *Medicine Update*, Bichile SK (ed), Mumbai. Association of Physicians of India. **Vol. 18:**(i)-(ii)
2. Johansson BB (2000). Brain plasticity and stroke rehabilitation. *Stroke* **31**: 223-230.
3. Okano H (2002). Stem cell biology of the central nervous System. *J. Neurosci Res* **69**: 698 – 707.
4. Stem cell basics: National Institute of Health. **http://stem** cells.nih.gov /infocenter / stem cell basics.asp.
5. Zivin JA (2000): Cell transplant therapy for stroke. Hope or Hype. *Neurology* **55**: 467.
6. Savitz SI, Rosenbaum DM, Dinsmore JH *et al* (2002). Cell transplantation for stroke. *Ann Neurol* **52**: 266 – 275.
7. Bjorklund A, Lindvall O (2000). Cell replacement therapies for central neurons systems disorders. *Nature Neuroscience*. **3**: 537 – 544.

8. Issacson O, Deacon T (1997). Neural transplantation studies reveal the brain capacity for continous reconstruction. *Trends Neurosci* **20**: 477- 482.

9. Brevig T, Holgersson J, Widner H (2000). Xenotransplantation for CNS repair: immunological barriers and strategies to overcome them. *Trends Neurosci* **23**: 337 – 334.

10. Boer GJ (1999). Ethical issues in neurografting of human embryonic cells. *Theor. Med. Bioeth.* **20**: 461 – 475.

11. Cozzi E, White DJ (1995). The generation of transgenic pigs as potential organ donors for humans. *Nat Med* **1**: 964 – 966.

12. Jacoby DB, Lindberg C, Cunnigham MG *et al* (1999). Long term survival of fetal porcine lateral ganglionic eminence cells in the hippocampus of rats. *J Neurosci Res* **56**: 581 – 594.

13. Edge AS *et al* (1998). Xenogeneic cell therapy: Current progress and future development in porcine cell transplantation. *Cell transplantation* **7**: 525 – 539.

14. Patience C *et al* (1997). Infection of human cells by an endogenous retrovirus of pigs. *Nat Med* **3**: 282 – 256.

15. Staines WA, Morassutti DJ, Reuhl KR *et al* (1994). Neurons derived from P19 embryonal carcinoma cells have varied morphologies and neurotransmitters. *Neuroscience* **58**: 735 – 751.

16. Magmeson DSK, Morassutti DJ, Staines WA *et al* (1995). In vivo electrophysiological maturation of neurons derived from a multipotent precursor (embroymal carcinoma) cell line. *Dev Brain Res* **84**: 131 – 141.

17. Pleasure SJ, Lee VM (1993). N Tera 2 cells: a human cell line which displays characteristics of a human committed neural progenitor cells. *J Neurosci Res* **35**: 585 – 602.

18. Pleasure SJ, Page C, Lee VM *et al* (1992). Pure, post mitotic polarized human neurons derived from N Tera 2 cells provide a system for expressing exogenous proteins in terminally differentiated neurons. *J Neurosci* **12**: 1802 – 1815.

19. Guillemani I, Alonso G, Patey G *et al* (2000). Human NT2 neurons express a variety of neuro-transmission phenotypes *in vitro*. *J Comput Neurol* **422**: 385 – 395.

20. Hartley RS, Margulis M, Fishman PS *et al* (1995). Functional synapses are found between human N Tera 2 (NT2N, hNT) neurons grown on astrocytes. *J. Comput Neurol* **357**: 681 – 632.

21. Okano H (2002). Neural stem cells; progression of basic research and prospective for clinical application. *Keio J Med.* **51**: 115 – 128.

22. Svendsen CN, Caldwell MA (2000). Neural stem cells in the developing nervous system: implication for cell therapy through transplanatation. *Prog Brain Res* **127**: 13 – 34.

23. Nakamura Y, Sakakibara S, Miyata T *et al* (2000). The bHLH gene Hes1 as a repressor of neuronal commitment of the CNS stem cells. *J . Neurosci* **20**: 283 – 293.

24. Pencea V, Bingaman KD, Wiegand SJ *et al* (2001). Infusion of brain derived neurotrophic factors into the lateral ventricle of adult rat leads to new neurons in the parenchyma of the striatum, septum, thalamus, and hypothalamus. *J. Neurosci* **21**: 6706 – 6717.

25. Sanchez-Ramos JR (2002). Neural cells derived from adult bone marrow and umblical cord blood. *J. Neurosci Res* **69**: 880 – 893.

26. Sanchez – Ramos J, Song S, Cardozo – Pelaez F *et al* (2000). Adult bone marrow stromal cells differentiate into neural cells in vitro. *Exp Neurol* **164**: 247 – 256.

27. Woodbury D, Schwarz EJ, Prockop DJ *et al* (2000). Adult rat and human bone marrow stromal cells differentiate into neurons. *J. Neurosci Res* **61**: 364 – 370.

28. Sanchez – Ramos J, Song S, Kamath SG *et al* (2001). Expression of neural markers in human umbilical cord blood. *Exp Neurol* **171**: 102 – 115.

29. Koji A (2000). Therapeutic potential of neurotrophic factor and neural stem cells against ischaemic brain injury. *J. Cereb Blood Flow Met* **20**: 1393 – 1408.

30. Nishino H, Borlongan CV (2000). Restoration of function by neural transplantation in the ischaemic brain. *Prog Brain Res* **127**: 461 – 76.

31. Eriksson PS, Perfilieva E, Bjork – Ericcsson T *et al* (1998). Neurogenesis in adult human hippocampus. *Nat Med* **4**: 1313 – 1317.

32. Hodges H, Sinden J, Meldrum B, Gray J(1994). In: Functional Neural Transplantation. Dunnett B, Bjorklund A. eds. Raven, New York 347 – 386.

33. Hodges H *et al* (1996). Contrasting effects of fetal CA1 & CA3 hippocampal grafts on deficits in spatial learning and working memory induced by global cerebral ischaemia in rats. *Neuroscience* **72**: 959 – 988.

34. Sorensen JC, Grabowski M, Zimmer J *et al* (1996). Fetal neocortical tissue blocks implanted in brain infarcts of adult rats interconnect with the host brain. *Exp Neurol* **138**: 227 – 235.

35. Mattsson B, Sorensen JC, Zimmer J *et al* (1997). Neural grafting to experimental neocortical infarcts improves behavioral outcome and reduces thalamic atrophy in rats housed in enriched but not in standard environments. *Stroke* **28**: 1225 – 1232.

36. Sotelo C, Alvarado – Mallart, RM (1991). The reconstruction of cerebellar circuits. *Trends Neurosci* **14**: 350 – 335.

37. Chen J, Li Y, Wang L *et al* (2001). Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischaemia in rats. *Stroke* **32**: 1005 – 1611.

38. Chem J, Sanberg PR, LiY *et al* (2001). Intravenous administration of human umblical cord blood reduces behavioral deficits after stroke in rats. *Stroke* **32**: 109 – 115.

39. Mudrick LA, Bainbridge KG (1991). Hippocampal neurons transplanted into ischaemically lesioned hippocampus: anatomical assessment of survival, maturation and integration. *Exp Brain Res* **86**: 233 – 247.

40. Aoki H, Onodera H, YaeT *et al* (1993). Neural grafting to ischaemic CA1 lesions in rat hippocampus : an autoradiographic study. *Neurosci* **56**: 345 – 354.

41. Grabowski M, Johansson BB, Brundin P (1995). Neocortical grafts placed in the infracted brain of adult rats: few or no efferent fibres grow from transplant to host. *Exp Neurol* **134**: 273 – 276.

42. Nishino H, Koide K, Aihara N *et al* (1993). Striatal grafts in the ischaemic striatum improve pallidal GABA release and passive avoidance. *Brain Res Bull* **32**: 517 – 520.

43. Borlongam CV, Tajima Y, Trojanowski JQ *et al* (1998). Transplantation of cryopreserved human embryonal carcinoma-derived neurons (NT2N cells) promotes functional recovery in ischaemic rats. *Exp. Neurol* **149**: 310 – 321.

44. Veizovic T, Beech JS, Stroemer RP *et al* (2001). Resolution of stroke deficits following contralateral grafts of conditionally immortal neuroepithelial stem cells. *Stroke* **32**: 1012 – 1019.

45. Li Y, Chopp M, Chen J *et al* (2000). Intrastriatal transplantation of bone marrow non hematopoetic cells improves functional recovery after stroke in adult mice. *J Cereb Blood Flow Metab* **20**: 1311- 1319.

46. Chen J, Li Yi, Wang L *et al* (2001). Therapeutic benefit of Intracerebral transplantation of bone marrow stromal cells after cerebral ischaemia in rats. *J Neurol Sci* **189**: 49 – 57.

47. Zhao LR, Duan WM, Reyes M *et al* (2002). Human bone marrow stromal cells exhibit neural phenotypes and ameliorate neurologic deficits after grafting into the ischaemic brain of rats. *Exp Neurol* **174**: 11 – 20.

48. Li Y, Chen J, Wang L *et al* (2001). Treatment of stroke in rat with intracarotid administration of marrow stromal cells. *Neurology* **56**: 1666 – 1672.

49. Nelson PT, Kondziolka D, Wechsler L *et al* (2002). Clonal human (hNT) neuron grafts for stroke therapy. Neuropathology in a patient 27 months after implantation. *Am J Pathol* **60**: 1201 – 1206.

50. Hadani M, Freeman T, Munsiff A *et al* (1992). Fetal cortical cells survive in focal cerebral infarct after permanent occlusion of the middle cerebral artery in adult rats. *J. Neurotrauma*. **90**: 107 – 112.

51. Grabowski M, Johansson BD, Brundin P (1994). Survival of fetal neocortical grafts implanted in brain infarcts of adult rats: the influence of post lesion time and age of donor tissue. *Exp Neurol* **127**: 126 – 136.

52. Kondziolka D, Wechsler L, Achim C (2002). Neural transplantation for stroke. *J Clin Neuroscience* **9**: 225 – 230.

53. Kondziolka D, Wechsler L, Goldstein *et al* (2000). Transplantation of cultured human neuronal cells for patients with stroke. *Neurology* **55**: 565 – 569.

54. Meltzer CC, Kondziolka D, Villemagne VL *et al* (2001). Serial (18F) flourodeoxyglucose position emission tomography after human neural implantation for stroke. *Neurosurgery* **49**: 586 – 591.

55. Kondziolka D, Wechsler L, Tyler-Kabara E (2002). The role of cell therapy for stroke. *Neurosurgery focus* **13 (5)**: 1 – 6.

56. Oh Young Bang Hyu Lee *et al* (2005). Autologous mesenchymal stem cell transplantation in stroke patients. *Annals of Neurology* **57(6):** 874-882.