Annals of the National Academy of Medical Sciences (India)

Volume 52, No. 2
April-June, 2016
ISSN 0379 - 038X

Annals of the National Academy of Medical Sciences (India)

-: A quarterly Journal :-

Annual Subscription Rates

Inland	Rs. 500.00
Foreign	$ 30.00
	£ 15.00
Single Copy	Rs. 150.00

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Ann Natl Acad Med Sci (India), **52(2):** 76-138, 2016

ANAMS 52 (2): 76-138, 2016

CONTENTS

Editorial

Medical Discoveries: From benchtop to bedside

Long ago, when for the first time Edward Jenner, a country doctor living in Berkeley (Gloucestershire), England, who performed the world's first vaccination in 1796, it leads to speculations that all the disease can be prevented or eradicated. With unifying efforts of Biomedical scientists along with public health workers, it was only in 1980 the small pox was ultimately eradicated from the World. Thus it took nearly 2 centuries to see the fruits of the untiring work of our researchers and healthcare manpower.

Tuberculosis, a disease considered to be incurable before 1946, found a ray of hope with discovery of Streptomycin — an antibiotic purified from *Streptomyces griseus* — as the first antibiotic with proven activity against Mycobacterium tuberculosis. However, biomedical scientists found that uncertainties remained about its ability to consistently cure patients, and this was closely followed by the realization that drug resistance develops rapidly when a single agent is used for the treatment of TB. The untiring efforts of scientists once again lead to development of another drug Rifampicin in 1960s which changed the scenario of treatment of TB in sanatorium to domiciliary mode. It also paved way for the scientists to realize that only randomized controlled trials are the gold standard for judging the efficacy of a new drug.

The science fictions give us an inkling that it is not impossible to think about innovative device drug or idea to change the face of healthcare. However, life of scientists working on these innovations provide us teaching tips that scientific discoveries are not abrupt but took pretty long time for fruitful translation into the clinical practice.

When a four-year old girl became the first gene therapy patient on September 14, 1990 at the NIH Clinical Center as she had adenosine deaminase (ADA) deficiency, a genetic disease which leaves her defenseless against infections, it was speculated that all genetic disorders may be cured with help of Gene therapy. Twenty five years later we are still not able to develop an effective and safe gene therapy for as common a disorder as Thalassemia.

There are ideas, there are researcher devoting their lifetime on these ideas, there are scientists who have proved others wrong and there are others who created examples of ethical medical practice using and there is generation of medical scientists using evidence based medicine in daily practice and who are looking forward to new

discoveries and inventions. But one thing to be realized is that there is no short cut for finding cure to human diseases.

In this issue, we can visualize the long journey for the infectious diseases namely Neurocysticercosis and challenges in development of vaccine for Japanese encephalitis.

It is also heartening to note that innovations are not only needed in health care devices but also in way we prepare our future generation of physicians. Innovations are also required for detecting diseases at the nascent stage. Then there are benign breast diseases where life time efforts leads to elucidate the natural history and development of cost effective therapy. This issue is dedicated to the efforts of all biomedical scientists whose untiring work and efforts culminated in better bedside therapies and whose teaching will prove lighthouse for future generation of Indian biomedical scientists.

Dr Sanjeev Misra

Ann Natl Acad Med Sci (India), **52(2):** 76-99, 2016

Neurocysticercosis – A Journey from Pre-independence to Modern India

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ABSTRACT

Neurocysticercosis (NCC) is infestation of the human brain by the larva of worm, *Taenia solium* and is the most prevalent central nervous system (CNS) helminthiasis. The disease is widespread in tropical and subtropical regions of the world, including the Indian subcontinent, China, Sub-Saharan Africa, Central and South America and contributes substantially to the burden of epilepsy in these areas(1) . CNS involvement is seen in 60-90% of systemic cysticercosis. About 2.5 million people worldwide are infected with *T. solium*, and antibodies to *T. solium* are seen in up to 25% of people in endemic areas(1-3) . A higher prevalence of epilepsy and seizures in endemic countries is partly because of a high prevalence of cysticercosis in these regions. Seizures are thought to be caused by NCC in as many as 30% of adult patients and in 51% of children in population based endemic regions (2) . About 12% of admissions to neurological services in endemic regions are attributed to NCC and nearly half a million deaths occurring annually worldwide can be attributed directly or indirectly to NCC (Bern *et al*.). Punctate calcific foci on CT scan are a very common finding in asymptomatic people residing in endemic areas, found in 14-20 % of CT scans. Both seizures and positive cysticercus serology are associated with the detection of cysticerci on CT scans. Seroprevalence using a recently developed CDC- based enzyme-linked immunotransfer blot (EITB) assay is estimated at 8-12% in Latin America and 4.9-24% in Africa and South-East Asia. It is estimated that 20 million people harbour neurocysticercosis worldwide(1) .

Key words : Neurocysticercosis (NCC), SCG, T. Solium, cysticerci.

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DR. BALDEV SINGH ORATION delivered during NAMSCON 2015 at the All-India Institute of Medical Sciences, Patna.

INTRODUCTION

Neurocysticercosis is the main cause of acquired (late-onset) epilepsy in many resource- poor countries(4). The clinical presentation, course and outcome of NCC depends on clinical subtype of NCC, which in turn is determined by several factors : the anatomical location of the cysticerci (parenchymal NCC, subarachnoid-racemose cysticerci, ventricular cysticerci, spinal cysticerci), number of cysticerci (single, few, disseminated) and evolutionary stage of the cysticerci(5). The latter, i.e., evolutionary stage is based on pathological classification of cysticercosis but has been adapted for use in clinical and imaging studies(6-7). The vesicular stage refers to the live or cystic or active stage of the cysticercus. The colloidal stage is also called transitional or degenerating stage. Likewise, the granular nodular stage represents the dying parasite. The fibro-calcified stage represents the dead or inactive parasite. The varied presentations of NCC are due to the several different combinations of location, number and stage of the cysticerci. For instance, multiple NCC, especially when the number of cysts is in 100s presents with dementia and raised intracranial pressure (8-9). When multiple parenchymal cysticerci begin to degenerate, the clinical presentation is one of raised intracranial tension, seizures and focal neurological deficits. On the other hand, when only solitary or few cysticerci degenerate, the clinical presentation comprises of few seizures, that are usually easily controlled(10). These single or few cysticerci represent a relatively benign form of NCC. Other manifestations of NCC include communicating hydrocephalus, stroke-like presentation and cranial nerve deficits in the case of subarachnoid-racemose cysticercosis and obstructive hydrocephalus in the case of intraventricular NCC (11-12).

The lack of basic sanitation, contamination of available drinking water and crowded living conditions, often with animals living side by side- all of which render people at risk for developing cysticercosis in resource- poor regions of the world. In community-based studies in endemic regions, seizures are associated with pig-raising and positive cysticercus serology(13). Due to the epidemiology of the disease, it is particularly common in the slums.

Long-held concepts regarding the global burden of the disease, its morbidity and mortality and its prevalence in endemic areas have changed considerably following developments in the fields of epidemiology and neuroimaging. A major false assumption was the belief that NCC occurs only after eating raw or contaminated pork(14). It is now believed that any uncooked food may be contaminated with ova of *T. solium*. Contamination is known to occur when raw vegetables grown in land fertilised with human faeces are ingested, or when people harbouring tapeworms prepare food with hands containing the ova. This is borne out by the fact that areas with tradition of good sanitation do not report a high incidence of NCC, but regions with

less developed sanitation systems may have as much as 10% prevalence of cysticercosis.

As computed tomography (CT) and magnetic resonance (MR) imaging began to be routinely used in patients suffering from seizures, it became apparent that the prevalence of NCC was much greater than previously thought. Epilepsy was not known as a frequent symptom of NCC in the pre-CT era except for Dixon and Lipscomb's early study on British soldiers stationed in India. Definitive diagnosis of this condition could only be made *post mortem* until the use of imaging techniques coupled with serologic assays for the detection of parasite antigen in blood and cerebrospinal fluid (CSF). These methods enabled easy and accurate diagnosis of NCC in a large proportion of people with epilepsy, and led to recognition of this disease as a major public health problem worldwide.

Economic costs of NCC include costs of medical treatment, lost working days and loss due to livestock condemnation. In a study from India, the authors estimated the direct cost per patient with NCC treated with AEDs for the period until resolution of lesion to be Rs. 5916 or 41.4% of GNP per capita(15). Total costs including indirect costs were calculated to be Rs. 7273 per patient or 50.9% of GNP per capita. Based on these estimates, annual costs due to NCC in India have been pegged at Rs.104 million/year. In Mexico the corresponding figure stands at Rs. 3560 million and in Brazil at Rs.3400 million/year. Due to increasing migration into affluent countries from endemic areas, these nations also face a substantial disease burden: 10% of seizures in the South-Western USA are due to NCC and the annual loss due to NCC in the USA has been calculated to be US$ 8.8 million.

The most important aspect of NCC prevention and control is proper sanitation and maintenance of personal hygiene(16-17). Scientific pig rearing measures with proper feeding, antihelminthic treatment to pigs, and pig vaccination are being investigated as methods of eliminating *T. solium* from endemic communities. Inspection and proper cooking of pork is essential to prevent development of the carrier stage. Carriers may be treated with niclosamide or single dose praziquantel.

Taenia solium: Life Cycle

Man is the only definitive host for *T. solium*, the adult worm which inhabits the human jejunum and ileum. However, the developmental cycle of the parasite includes a larval stage in intermediate hosts, which are usually pigs but may by accident also be human beings (Fig. 1) (5,18). Ova are liberated into the environment per anum in the form of egg-containing segments (gravid proglottids) and shed from the body of the worm which may, by means of contaminated food or water, be ingested by another human host. The definitive host may also be infected through faecal-oral contamination as a result of poor hygiene and also by reverse peristalsis, which leads to the migration of ripe proglottids to the stomach. In either

case, the eggs rupture to liberate hexacanth larvae (oncospheres) which burrow into the gastric mucosa and reach the portal venous system. From here, they are distributed across the blood stream to various organs. Once they reach small terminal vessels, the embryos establish and encyst to form the larval vesicles or cysticerci, reaching their definitive size in 2-3 months. They are then disseminated to various organs, preferentially to more vascular structures such as the ones like skeletal muscle, brain and eye. In the brain, parenchymal involvement is more common than extraparenchymal. Outside the nervous system, cysticercosis causes few symptoms as the parasitic cysts are destroyed by the host's immune response. Symptoms or signs usually result from parasites located in the eye or the nervous system. Pigs are infested by eggs or proglottids in the stools of a human tapeworm carrier.

The number of ingested ova, duration of exposure, and viability of ova influence the host response, clinical features and the course of the disease. Once lodged in host tissue the contents of the oncospheres liquefy leading to formation of the *cysticercus*.

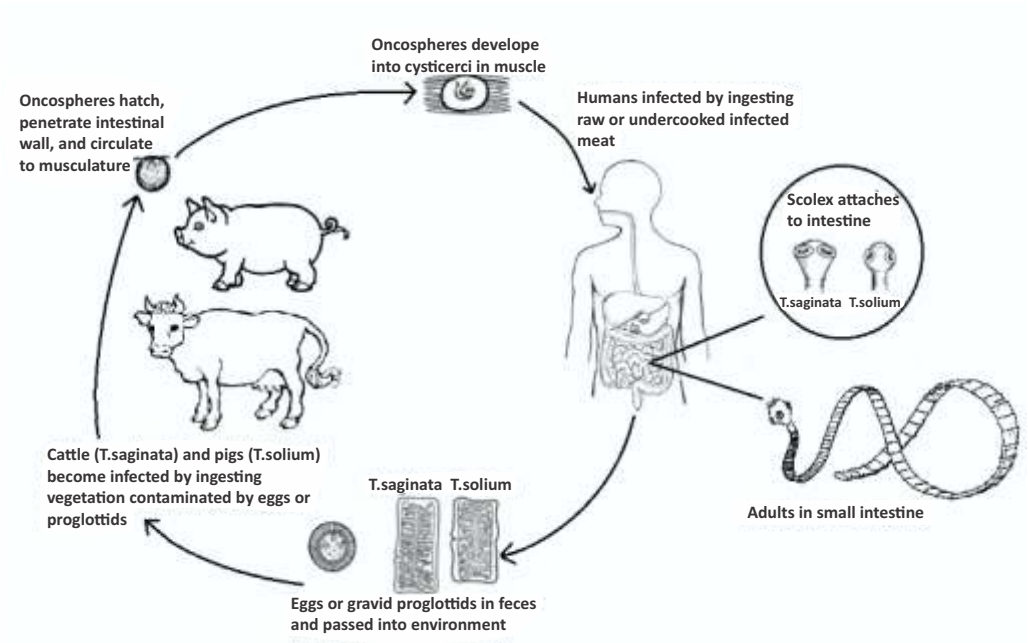

Fig. 1 : Figure depicting the life cycle of Taenia solium, going through various stages from egg to oncospheres to adult

Clinical Manifestations :

Symptoms and signs of NCC infection of the human brain are several and varied(19-20) . These may be delayed for years or may remain subclinical and depend on the host response, location number and size of the cyst/s(19). The mean duration between infection and presentation is 10.7 months (range 6-27 months) (11). Epilepsy is major problem in resource poor countries (with a prevalence of 5-10/1000 people), largely because of the high prevalence of NCC in these areas(2, 4) . Following the advent of CT, Latin American studies have shown that over 30% of epilepsy, and over half of all late-onset seizures are related to NCC(2,4,21). Parenchymal NCC commonly presents with seizures (up to 90%) and headaches (11, 22). Seizures may be single, clustered or recurrent. They are either focal with or without secondary generalization or may be generalized at onset. Extraparenchymal cysts are usually not associated with seizures and these patients most commonly present with hydrocephalus, arachnoiditis, cranial nerve palsies and stroke-like episodes(12, 22, 23).

In children too, seizures have been reported to be the commonest manifestation of NCC (65.5%), followed by headache (37.9%) and focal neurological deficits (24). Around 26-72% of children with a first seizure have a ring or disc lesion on CT, which in most cases represents SCG (25).

Seizures may occur in any stage of NCC, but acute symptomatic seizures occur when a parenchymal cyst starts degenerating (symptomatic of the acute inflammatory response), and chronic epilepsy is perhaps due to gliosis around a chronic calcific lesion. Thus, seizures could occur due to several reasons in NCC. In resource poor countries, NCC is probably the commonest cause of acute symptomatic seizures; 92% of Indian patients with recurrent acute symptomatic seizures were due to NCC in one series (26). It has been proposed that seizures in the context of a degenerating cyst with oedema be considered acute symptomatic seizures, while those occurring in persons with calcified cysticerci are unprovoked (18).

Classification of NCC:

It is important to classify NCC as treatment and prognosis depends upon the classification. One way of classifying NCC is according to the pathological stages described by Escobar (6). Once a cysticercal cyst lodges in brain parenchyma, it undergoes spontaneous involution over varying periods. A parenchymal cyst typically goes through four morphological stages of involution: (a) vesicular; (b) colloidal; (c) granular-nodular and (d) calcific. This entire process may take between few weeks and several years.

Patients may also be classified into those with active infection (with live cysticercus), transitional form (degenerating cyst) or inactive disease

(dead parasite), although they frequently have multiple cysts simultaneously in varying stages of involution (7). Those patients with inactive infection may show calcification on CT, plain X-ray or MR: the former is thought to be most sensitive. They typically have a history of seizures in the past. However, the presence of such calcific foci is a known risk factor for seizure recurrence. Active parenchymal neurocysticercosis represents over 60% of cases in most series, presenting predominantly with seizures (22). On imaging, most patients show evidence of inflammation around the cysts in the form of oedema or enhancement of the cyst wall. Finally, depending upon the site of involvement, NCC may be classified into cranial (parenchymal, ventricular, subarachnoid – cisternal), spinal and mixed.

Diagnosis of Neurocysticercosis :

Accurate diagnosis of NCC is possible after interpretation of clinical data together with findings of neuroimaging studies and results of immunologic tests.

Imaging Diagnosis:

Computed tomography (CT) and magnetic resonance imaging (MRI) provide objective evidence about the topography of lesions, then evolutionary stage and degree of the host inflammatory response against cysticerci. CT is superior for detection of calcific lesions, whereas intraventricular, subarachnoid cysts and peri-cystic inflammation are better delineated by MRI. The intraventricular form presents with obstructive hydrocephalus, and only indirect signs like enlargement or deformation of ventricles or basal cisterns are seen on CT. MRI is the most accurate technique for imaging cysticercosis, providing the best evaluation of the degree of infestation, location and evolutionary stage of the parasite. MRI is more sensitive than CT scan for the diagnosis of neuro-cysticercosis, since it better recognizes the perilesional edema and the degenerative changes of the parasite, as well as small cysts or those located in the ventricles, brain stem, cerebellum, or the eyes, and the racemose vesicles at the level of the posterior fossae and basal cisterns. CT scan, however, is more sensitive for the detection of calcifications.

Most parenchymal lesions appear and show enhancement on contrast injection, usually surrounded by oedema. The vesicular stage, with a viable larva, appears as a smooth thin-walled cyst that is CSF-like on CT and MR images with no oedema or contrast enhancement. A mural nodule is often present that represents a viable larval scolex. When cyst degeneration begins (colloidal stage) and host inflammatory response ensues, pericystic oedema and cyst wall enhancement are present. Enhancement implies disruption of the blood-brain barrier. Cyst fluid is hyperintense compared to CSF on MR imaging during this stage. The colloidal stage has a thicker enhancing wall enclosing cyst fluid and prominent oedema. In the healing or granular nodular stage, nonenhanced CT

scans show an iso-attenuated cyst with a hyperattenuated calcific scolex. Surrounding oedema is still present, and enhancement following contrast material administration persists. The residual cyst is isointense to the brain parenchyma on T1- weighted images and it is iso- to hypointense on T2-weighted images. Nodular or micro-ring enhancement is common at this stage, suggesting granuloma. Occasionally, a "target" appearance is seen with the calcified scolex in the centre of the mass. In the quiescent or residual stage, small calcified nodules without mass effect and usually without enhancement are seen.

Advantages of MRI over CT include: (a) capability of testing in different planes (axial, coronal, sagital); (b) it does not use iodine contrast and thus subjects are less prone to allergic reactions; (c) better sensitivity, except for calcifications; (d) it has various functional protocols (T1, T2, FLAIR, etc.) that allow for a better characterization of the parasite and of its evolutionary stage, and (e) no risk of radioactivity exposure.

Serological Diagnosis :

The enzyme-linked immuno-electrotransfer blot assay (EITB, Western Blot) provides specific confirmation of the diagnosis of NCC (27). In brief, this assay utilizes seven purified *T. solium* glycoprotein antigens (diagnostic bands GP50, GP42-39, GP24, GP21, GP18, GP14, and GP13) in an immunoblot format to detect infection-specific antibodies in serum or CSF samples. Reactions to at least one band are considered positive. Specificity of this assay is 100%, and its sensitivity in cases with viable parasites is estimated to be over 95% although it may be significantly lower in patients with a single cyst or a single degenerating parasite (28). Serologic tests are a valuable complement to neuroimaging in the evaluation of patients with suspected NCC, but they should not be used alone to exclude or confirm the diagnosis.

Solitary Cysticercus Granuloma :

A single enhancing CT lesion measuring less than 20 mm is a common finding upon CT of the brain of patients with seizures in India (29, 30). This lesion represents a solitary cysticercus granuloma (SCG) in the acute encephalitic phase. Epileptic seizures are by far the most common clinical manifestation of the SCG. While there is an over-abundance of reports of patients with SCG from India, these lesions have been reported from all over the world. In a hospital-based study from South India, this lesion accounted for 26% of etiological factors of symptomatic localization-related seizures (31) and 50% of etiological factors for acute symptomatic seizures. The SCG is one of the commonest form of NCC. Its true incidence in comparison to other forms of NCC is not clear. In the hospital-based studies the reported frequency of solitary cyst (either granuloma alone, or SCG and solitary live cysts taken together) varied between 3.5% to 43% (32). In a study from Ecuador study, this lesion

represented the single most common form of presentation of NCC, accounting for 23% of the cases (33).

Historical Perspective :

The solitary, small enhancing lesions , with surrounding edema were first reported as *microtuberculomas or immature tuberculomas* , by Bhargava and Tandon (34). There was no histological diagnosis at that time. Similar CT lesions were reported by some other Indian authors also. Wadia and colleagues also suggested similar lesions as *microtuberculomas* on the basis of a study on 39 patients with single small enhancing CT lesions (SSECTL), of whom ten patients had pulmonary tuberculosis (35). On this basis they advocated anti tubercular therapy for all patients with SSECTL. The diagnosis of micro-tuberculomas was first challenged by Sethi and colleagues (36), when they reported spontaneous resolution of lesions in patients with seizures, who did not take antitubercular treatment. These lesions were then described as *Vanishing or Disappearing lesions* in many reports (29, 37). Few authors considered these lesions as post-ictal as a result of breakdown of the blood brain barrier (29). Rajshekhar and colleagues performed stereotactic biopsy in patients with SSECTL and found evidence of cysticerci or merely parasitic material in several biopsy materials (38-41). The authors concluded that the majority of SSECTLs were solitary cysticercus granulomas (SCG). Subsequently, they evolved a set of diagnostic criteria for an initial diagnosis of SCG. These diagnostic criteria were found to have a sensitivity of 99.5 %, specificity of 98.9%, positive predictive value of 99 %, and negative predictive value of 99.5 % (42). Diagnostic criteria were further elucidated in a consensus paper (Table 1).

Table 1.Clinical and radiologic features consistent with diagnose of SCG

A. Clinical Feature that are supportive of diagnosis of SCG
   Focal seizures or without secondary generalization
   Note: Seizures may be new onset or of longer duration may be generalised at onset may occur in clusters (2 or more seizures over 2-3 days) may be flowed by unilateral or diffuse headache lasting for a few hours to day/s or may be followed by transient and mild postictal neurological deficit.
B. Clinical features that make a diagnosis of SCG unlikely
   Persistent and severe neurological deficit
   Clinical evidence of intracranial hypertension
   Evidence of neurologic disorder, other systemic disease (i.e systemic infection such as AIDS),that can account for imaging findings.
   Age < 2 years and > 60 years
C. CT-features compatible with diagnosis of SCG (see Fig. 1)
   Single small (<20 mm) well defined
   Contrast -enhancing (closed ring disc or nodular type)

With or without surrounding edema
Associated with minimal mass effect and no midline shift

D. MRI features compatible with diagnosis of SCG (see Fig. 1)
Single small (< 20mm lesion with fluid contents)
T1 sequence intensity slightly greater than or isointense of CSF
T2 sequence hyperintense or isohypointense with central hyperintensity
Ring or nodular type enhancement after contrast
Scolex may or may not be visible as an eccentric nodule within the fluid cyst contents
(T1 isointense and T2 iso-hypointense)
Mild to moderate surrounding edema but no midline shift

Abbreviation SCG- Solitary cysticercus granuloma

These criteria were adapted from a review article, "A Diagnostic and therapeutic scheme for a Solitary Cysticercus Granuloma" (Neurology 75, December 14, 2010)(43).

Treatment of Neurocysticercosis :

Antihelminthic Therapy :

Although albendazole and praziquantel have been available since the 1980s and are relatively safe drugs with minimal toxicity and brief dosage schedules, even today there is considerable debate about their role in the treatment of NCC (44, 45). Anecdotal reports of the efficacy of the isoquinoline derivative praziquantel in NCC were available from the early 1980s, and an uncontrolled clinical trial in 1985 suggested its usefulness in patients with viable parenchymal cysticerci (46). Similarly, albendazole, a benzimidazole derivative has been used for human NCC since 1988 (47).

A head-to-head comparison between the two antihelminthic agents showed albendazole to be slightly superior, to have better penetration into the brain, and to cost less (47). The usual 30-day duration of treatment (15mg/kg/day in 2 divided doses) is based on prior experience with human echinococcus, but serial MRI studies have demonstrated an equal benefit with an 8-day course (48) . Various studies have demonstrated equal efficacy with 15- and 17-day courses of albendazole, the outcome being measured by the change in cyst number and morphology on successive contrast-enhanced CT scans (49). On cysticidal treatment including both praziquantel and albendazole, the problem is that patients may develop headache or vomiting during treatment due to the strong inflammatory reaction elicited in the brain due to larval degeneration induced by the anti-helminthic agent. In one study conducted on patients with multiple cerebral cysticerci, adverse effects including nausea, vomiting and abdominal pain were seen in 38% of patients receiving albendazole. These were more severe with longer therapy. Headache was seen in 90% of patients at baseline and in 92% during

therapy. 24% had seizures during treatment, all of which had prior history of seizures. Anti-inflammatory drugs and corticosteroids have been used widely to manage the antihelminthic drug-induced exacerbations. However, the routine use of steroids is not recommended, particularly as concomitant steroid administration reduces plasma levels of praziquantel. They may be given if the patient develops symptom or signs of intracranial hypertension during treatment.

As noted above, the effect of cysticidal therapy on the course and outcome of the disease has been a matter of considerable debate. The possible outcomes from the host parasite interaction in NCC include continued growth of the parasite, eventually producing a giant cyst, death of the cyst with resolution or calcification, and immune-mediated damage to the CNS parenchyma leading to neurologic symptoms and sequelae (50). Four main arguments have been put forth to support the view that cysticidal treatment is at best ineffective in preventing further seizures, and may even lead to clinical deterioration. First, the sudden destruction of parasites engendered by treatment may trigger an inflammatory reaction resulting in headache and seizures acutely, or an excess of gliosis in the long run leading to more seizures. Second, in a considerable fraction of patients with NCC, the disease is either clinically-silent or produced only mild symptoms (e.g., occasional seizures), which are easily managed. Third, in some patients the disease will be adequately eliminated either by host's response or spontaneous regression without the need for therapeutic intervention. Finally and fourth, the physical elimination of the parasite does not necessarily mean that the patient's neurologic dysfunction would improve. Against this has to be weighed the fact that therapy is safe, effective and convenient?

In 2004, the results of a randomised, placebo-controlled trial of albendazole in cystic parenchymal NCC were reported (51). This trial proved to be a benchmark in clinical trial research in NCC with its optimal study design, selection of outcome parameters and sound analysis. The study revealed that with treatment with albendazole, more patients demonstrated resolution of their lesions at six months post-treatment. At six months, 37% in the treated group were free of active lesions in comparison to 14% in placebo group. In addition, fewer patients in the treated group had partial seizures and significantly fewer patients had seizures with secondary generalization over a two-year period. Between 2 and 30 months of follow-up, 33% in the treated group in comparison to 48% in the placebo group had partial seizures (not significant). In the same period, 22% in the treated group had seizures with secondary generalisation in comparison to 37% in the placebo group ($p=0.003$). Thus, treatment with albendazole resulted in a reduction in the number of seizures and this effect was significant for seizures with secondary generalization. The authors of this study underscored the benefits of treatment with the argument

that generalised seizures had a more devastating impact on the lives of the patients than partial seizures and hence, their reduction was a worthwhile outcome of treatment.

The trial demonstrated the benefits of treatment, these can best be construed to be modest and restricted to seizures with secondary generalisation (51). It may be argued that seizures, whether partial or generalised and the occurrence of either partial or generalised seizures precludes an individual from driving in most countries. The trial demonstrated that even after treatment with albendazole; about 2/3rds of the patients receiving albendazole continue to harbor active cysts, new drugs or different treatment regimens need to be explored for this majority of patients with NCC. Probably, studies are also required in order to understand why albendazole seems to work in some cases and why it does not in others. Appealing options include newer drugs (oxfendazole, for instance), combination of several anti-parasitic agents, either simultaneously or sequentially, and combination of anti-parasitic agents with immunosuppressive agents.

Apart from these trials a number of other clinical trials have evaluated a variety of treatment regimens of albendazole and praziquantel. For instance, a seven-day course of albendazole has been compared with longer durations of treatment. A single-day treatment regimen with praziquantel was also evaluated. It was found to be of modest benefit in solitary cysticercus granuloma and not effective in multiple neurocysticercosis. Although, a large number of trials with antihelminthic treatment can be found in published literature, no firm conclusion can be derived from these early studies. Many of the studies had small number or subjects, were unrandomised or uncontrolled or used historical controls only. Other methodological problems have vitiated results in some studies, such as inclusion of cysts in various stages of development or of patients with chronic epilepsy and a lack of a clearly defined outcome.

In a meta-analysis, Otte and colleagues identified 10 randomised controlled trials of anti-parasitic treatment in patients with 1-2 enhancing lesions, involving 765 subjects, though there was considerable heterogeneity in the various studies regarding duration of antihelminthic (3-28 days), frequency of follow up and the radiological response (52). One single study compared single day praziquantel treatment as compared with placebo and demonstrated higher rate of resolution at 3 months. Though the placebo arms also demonstrated resolution of lesions at one year with symptomatic therapy alone but the rate of resolution was faster with albendazole. A single study reported 12 months follow up and no difference in seizure freedom. In the five studies, which reported follow up at 6 months, 180/199(90%) were seizure free in the albendazole arm compared to placebo. Overall there was no significant difference in the frequency of calcifications at the end of follow up.

It appears that no standard treatment can be recommended for NCC. The parasitic disorder comprises several different presentations and treatment approaches depend on the clinical presentation, which in turn is determined by the location (parenchymal, intraventricular, subarachnoid-racemose, spinal), number of cysts and evolutionary stage of the cysticerci. Live (or cystic or vesicular) parenchymal cysticerci should be treated (Level 1 evidence). Evidence in favour of treatment of degenerating cysticerci (in the colloidal or granular-nodular stage) is less convincing and probably needs further study. The calcified stage of cysticercosis does not require any antihelminthic treatment (Level 1 evidence). Treatment with anti-helminthic drugs is contraindicated in disseminated cysticercosis, cysticercotic encephalitis and ocular cysticercosis. In the former case, treatment may lead to fatal intracranial hypertension, while in the latter case, treatment may lead to blindness.

In a recent trial, combination treatment (Albendazole and Praziquntel) was associated with significantly increased proportion of patients with complete resolution (64% compared with 37% in standard albendazole group), which means a significantly improved rate of parasite clearance (53). The beneficial effects appeared to be restricted to people with multiple NCC. No difference in outcome was noted in people with single NCC.

Corticosteroids :

Corticosteroids have been used with a variety of indications in NCC. Benefits of their use are clearly discernable and often dramatic in some of the more severe forms of NCC such as cysticercotic encephalitis and subarachnoid-racemose cysticercosis. Furthermore, since the administration of albendazole often provokes seizures, focal neurological deficits and even intracranial hyper-tension in patients with parenchymal NCC due to the peri-cystic inflammation induced by cyst degeneration, it is routine clinical practice to co-administer corticosteroids with antihelminthic drugs. This practice of the co-administration of corticosteroids with albendazole is believed to reduce the incidence of adverse effects owing to their anti-inflammatory actions. As a result, nearly all clinical trials of albendazole in NCC (SCG included) have incorporated corticosteroids in their intervention arm. The co-administration of the two agents does not allow us to dissect the benefits derived due to corticosteroids alone from those due to albendazole.

Antiepileptic Drug Therapy :

Seizures in NCC are managed in a manner similar to seizures of other etiologies. Carbamazepine is favored for treatment of partial seizures due to NCC; phenytoin is another drug that is often used (54). Drug rash with phenytoin has been reported to be more frequent in patients with SCG, and clobazam was used as a replacement with adequate

seizure control (55). Another problem is that both carbamazepine and phenytoin induce the hepatic drug metabolizing enzymes that metabolize the anti-helminthic agents, albendazole and praziquantel. This might decrease levels of the antihelminthic agents, thereby potentially compromising their cysticidal efficacy. Follow-up studies from Latin America of patients with multiple parenchymal NCC suggested a high frequency of breakthrough seizures and of seizure recurrence (nearly 50%) following AED withdrawal after a period of seizure remission.

Treatment of SSECTLs / SCGs :

Single Small Enhancing CT Lesions (SSECTLs) are a common finding on computed tomography (CT) of young persons with new-onset seizures in India and also in several other parts of the world. It is currently believed that these lesions represent involuting cysticercus granuloma. In many ways, SSECTL / REL are a unique manifestation of neurocysticercosis (NCC). Although, these lesions have been noted in several geographical locations, they seem to be reported in large numbers from India. Thus, they may represent a geographically -dependant manifestation of NCC. They differ from lesions that have been studied in clinical trials described above in that they are encephalitic lesions as opposed to viable lesions. They also differ from lesions previously studied in clinical trials in being single as opposed to multiple. It has also been proposed that these lesions are biologically different from active and viable NCC by representing host inflammatory response to the embryonal stage of the parasite rather than to an established parasite.

Role of Antihelminthics :

Efficacy of antihelminthic drugs has been well established in live, vesicular parenchymal cysticercosis. The earliest RCT of albendazole in SCG could not find any difference in the frequency of resolution of the granuloma at 3 months among those who were treated with albendazole compared with those treated with nonspecific therapy. The previous meta-analysis of RCT's of albendazole in people with 1-2 parenchymal granuloma, reported in 2006, failed to demonstrate a better resolution rate with the use of albendazole (OR:1.18, 95%, CI: 0.82 - 1.71, P= 0.38). Many more RCTs have undertaken after this meta-analysis. In the recent meta-analysis of 10 RCTs by Otte *et al*., involving 765 subjects with SCG, of antihelminthic treatment (52, 56-65), some subjects had 2 rather than a single enhancing lesion in one study. This meta-analysis was different from the previous meta-analysis as it analysed pooled data according to the time of outcome assessment in the component RCT's (i.e 3,6 & 12 months). A significantly higher rate of granuloma resolution was observed with antihelminthic treatment from 3 and 6 months (OR: 2.09; 95% CI: 1.41–3.09; p = 0.0003; I^2 = 31%). When trials with repeated-measures design were excluded from analysis, the ORs for granuloma resolution remained increased in favour of antihelminthic treatment both at 3 months (OR: 2.25; 95% CI: 1.43–3.52; p =

0.0004; I^2 = 12% (data removed from 6 months) and 6 months (OR: 2.10; 95% CI: 1.28–3.47; p = 0.004; I^2 = 41%). As the chances of resolution clearly increase with longer follow up, therefore compared with the previous meta-analysis, this recent analysis established the benefit of anti-helminthic treatment with albendazole both at 3 and 6 months and in overall pooled analysis.

Seizure recurrence on antihelminthic treatment :

The proportion of subjects with seizure recurrence on antiepileptic drugs (AEDs) after therapeutic intervention with antihelminthic drugs was reported for 3-month follow-up in 2 RCTs (57, 61) , 6-month follow-up in 5 RCTs (58, 60, 61, 64, 65) and 12-month follow-up in 1 RCT (59). The proportion of subjects who remained seizure free was significantly higher in the antihelminthic treated group compared with controls for 3 months follow-up (non-event OR [i.e., 1/OR for seizure recurrence]: 4.05; 95% CI: 1.76–9.33; p = 0.001; I^2 = 0%), but not for 6 months (non-event OR: 1.79; 95% CI: 0.95–3.38; p = 0.45; I^2 = 0%) or 12 months (non-event OR: 0.64; 95% CI: 0.19–2.17; p = 0.47; heterogeneity not applicable). Meta-analysis of the pooled data from trials revealed significantly higher seizure-freedom rates at 3 and 6 months in the pooled antihelminthic-treated group compared with the pooled controls (non-event OR: 2.45; 95% CI: 1.49– 4.03; p = 0.0004), therefore in assessing seizures freedom also, the antihelminthic treatment was found to be beneficial.

Residual calcification with antihelminthic treatment :

Though the residual calcification is also associated with occurrence of long term seizures, these was no evidence of ether increased or decreased risk of residual calcification with antihelminthic treatment.

Conclusion :

In conclusion, the use of albendazole (with or without corticosteroids) is recommended in the treatment of SCGs because it offers increased possibility of seizure freedom in patients with SCGs and improve rates of granuloma resolution.

Role of corticosteroids :

The notion that corticosteroids alone could alter the clinical course and outcome of SCG arose out of anecdotal observations of their use in providing symptomatic benefit in patients with SCG. Symptoms such as headaches and seizure exacerbations have been found to resolve with the administration of corticosteroids. The routine use of oral corticosteroids alone (without specific antihelminthic treatment) in the management of SCG is advocated by certain authors. Otte *et al*., in their recent meta-analysis, included five RCTs, involving 457 people with SCG, of corticosteroid treatment (66-70).

In various separate clinical trials, the administration of either prednisolone (orally, 1 mg/kg/d for 10 days) alone (with

antiepileptic drugs) or intravenous methyl prednisolone (one gram/d for five days) was associated with clinical and/or radiological benefits over 6-9 month-periods (66-70). There were some discrepancies in the outcomes of these trials, but these appeared mainly due to the small size of the individual trials and when data from these trials were pooled beneficial effects were quite apparent. The administration of corticosteroids through either route resulted in significantly better rates of complete resolution of the SCG at six months and significantly fewer patients with seizure recurrence over a 6-9 months period. All these trials, however were located at one centre only and thus essentially represent a single-centre experience. Another shortcoming was that seizure recurrence was not clearly defined in these trials. Seizure recurrences in SCG are the result of brain parenchymal inflammation that follows cyst degeneration. The latter has been found to be a phasic rather than a continuous process. In keeping with this hypothesis, are the observations that seizures are often clustered in SCG and are related chronologically to the development of oedema surrounding, and of contrast enhancement of cysticercus lesions in the brain parenchyma, when followed over long periods of time. Hence the administration of corticosteroids may reduce the incidence of breakthrough seizures owing to their anti-inflammatory effects.

Granuloma resolution with corticosteroid treatment :

Five RCTs measured the effect of treatment with a short course of corticosteroids alone (standard AED treatment) on granuloma resolution (66-70). Three RCTs reported rates of granuloma resolution at 3 months (69, 70) and 3 at 6 months (66, 68, 70) of follow-up time. One RCT used a repeated-measures design with different modalities, CT at 3 months and MRI at 6 months (70). A random-effects analysis (performed because of significant heterogeneity; I^2 = 74%) did not reveal any significant difference in the rates of granuloma resolution over 3 and 6 months as well as in the overall combined analysis (OR: 1.82; 95% CI: 0.88–3.77; p = 0.11). This non-significant pooled effect remained using a sensitivity analysis when the repeated-measures data were excluded from the 3-month analysis (OR: 1.99; 95% CI: 0.79–5.04; p = 0.15) and the 6-month analysis (OR: 1.98; 95% CI: 0.78–5.00; p = 0.15).

Residual calcification with corticosteroid treatment :

Three RCTs of corticosteroid in SCG reported rates of residual calcification on follow-up imaging studies (67, 68, 70). The proportions of subjects with residual calcification on follow-up imaging were similar in the 2 pooled groups using fixed-effects analysis (OR: 1.18; 95% CI: 0.54–2.56; p = 0.68; I^2 = 0%). Subgroup heterogeneity was not significant (p = 0.25; I^2 = 25.7%).

Conclusion :

In conclusion, the pooled evidence does not confirm a beneficial effect of the administration of corticosteroids alone in the treatment of SCGs.

Corticosteroids alone versus Albendazole in conjunction with Corticosteroids :

Two open-labelled clinical trials compared the administration of albendazole with prednisolone with the administration of prednisolone alone (with antiepileptic drugs) for short periods of time upon presentation(61). In both trials, the rates of cyst resolution (defined as complete disappearance of the SCG) at six months were similar in the groups administered corticosteroids alone and corticosteroids and albendazole. The two trials were inconsistent in the differential effects of the two interventions on the number of patients with seizure recurrence on follow-up. One reported increased rates of seizures, whilst the other reported decreased rates of seizures with the administration of corticosteroids alone. These differences could be due to differences in trial methods and the small number of patients recruited in either trial. Nonetheless, these trials likewise underscore the need for a large clinical trial comparing the effects of corticosteroids alone versus the administration of corticosteroids with antihelminthic drugs. A trial of such design with adequate number of subjects alone could dissect out the beneficial effects of corticosteroids from that of antihelminthic drugs.

It is not clear whether the reduced risk of seizures was owing to a symptomatic anti-inflammatory effect of the corticosteroids or a long-term effect by altering the natural history of the granuloma. It is likely that corticosteroids had a long term modifying effect of the disease course in as much as CT performed at one month revealed significantly greater chances of complete resolution. It is known that the occurrence of seizures is related to the persistence of a lesion in the case of parenchymal NCC. Hence, the reduced incidence of seizures in the treated group might have been due to an earlier resolution of the granuloma with corticosteroid administration.

Whilst prescribing corticosteroid to patients with NCC, one should be guided by the basic principle of their administration, i.e., *"to achieve and maintain a satisfactory effect while using the lowest possible dose for the shortest time"*. One also needs to be aware of the drug interactions involved in such a therapeutic undertaking. Dexamethasone decreases praziquantel levels, potentially compromising its efficacy and thereby necessitating higher doses of the latter. On the other hand, dexamethasone decreases plasma albendazole-sulfoxide levels. Finally, antiepileptic drugs, including phenytoin and phenobarbitone induce the metabolism of corticosteroids. Consideration should also be made of the side-effects of corticosteroids; these include behavioural side effects and central serous retinopathy over the short term and diabetes, hypertension and bone loss over the long term.

Antiepileptic Drugs :

At least three short-term (up to one year follow-up), randomised studies have demonstrated that seizure recurrence rates are not much different between patients with solitary cysticercus granuloma administered short-duration (six months-one year) in comparison to longer duration (up to two years) of AED treatment (71-73). Longer duration (up to five years) seizure remission rates with short-term AED treatment have also been studied; these are about 85%, suggesting that seizure prognosis is essentially good (74). These follow-up studies have uniformly demonstrated that the single most important factor that determines seizure recurrence is the presence of a persisting lesion, whether a persisting involuting granuloma or a calcified residue. The likelihood of seizure recurrence in the event of complete resolution of the cysticercus granuloma is very low.

It seems reasonable to administer AEDs to patients with solitary cysticercus granuloma till such time that the lesion persists upon imaging studies. Once the lesion resolves completely, the AEDs can be safely withdrawn. In the case of residual calcification, longer duration of AED treatment may be required, but the duration of such treatment is not clear. Seizures in the setting of a calcified residue may occur owing to the intermittent release of antigenic material from within the calcified, dormant granuloma (75). This antigenic release causes acute symptomatic seizures due to cerebral irritation and manifests on imaging studies as contrast enhancement of the lesion and surrounding oedema. This may occur several years after the initial resolution of the granuloma. What proportion of calcific residues of cysticercus granulomas demonstrate this phenomena and for how long, remains to be settled by very long term follow-up studies.

The presence of perilesional gliosis on magnetisation transfer-MRI in the aftermath of a solitary cysticercus granuloma has been shown to be associated with seizure recurrence in one study (76). Residual gliosis demonstrated on either, magnetisation transfer imaging or fluid attenuated inversion recovery sequences may thus be a marker for seizure recurrences. The authors suggested that the demonstration of gliosis should dictate long-term AED treatment. Again longer duration, randomised studies of AEDs will be able to determine the need for, and duration of, AED therapy in such cases.

There is paucity of literature with regard to the choice of AEDs in NCC. Although, the range of options *a propos*, the choice of AEDs in epilepsy in general, also applies to NCC, it is desirable to administer an AED with a rapid onset of action. This is so because, seizures due to NCC are provoked by ongoing brain inflammation and are often clustered. Hence, there is a high risk of seizure recurrence in the immediate aftermath of a seizure. Thus, phenytoin-sodium in oral or parenteral loading doses is the preferred agent. However, one needs to be aware of

the adverse effects to this agent. In particular, we and other authors have reported a high incidence of anti-convulsant hypersensitivity syndrome in individuals with solitary cysticercus granuloma who were administered Phenytoin (55). A recent randomised, open-labelled trial demonstrated equivalent efficacy and lesser incidence of drug rash with clobazam treatment in comparison to phenytoin in patients with solitary cysticercus granuloma (77). However, the cost of treatment with clobazam is higher; this needs to be kept in mind, especially so whilst treating patients with NCC, a disease that largely affects the resource-poor sections.

Conclusion :

In conclusion, the risk of seizure recurrence remains high if there is persisting granuloma or resolves leaving behind a calcific residue. In this case, the longer duration of AED should be considered, though the exact duration of therapy is unclear. Any AED might be used for this recommended period of treatment in individuals with SCG. However a newer, non-enzyme-inducing AED might be considered for the period of time that antihelminthic treatment is administered.

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Ann Natl Acad Med Sci (India), **52(2):** 100-108, 2016

Japanese encephalitis virus: Uniqueness of immune response, vaccine development and future challenges

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ABSTRACT

Japanese encephalitis virus (JEV) is a major seasonal health problem in many rural areas in India and other parts of Asia. Transmission of virus is through mosquito vectors biting followed by peripheral multiplication site and exposed to host immune response before it succeeds in invasion of CNS. Thus protection from viral infection is a complex interplay of fight for superiority by virus and the host.

Cell mediated immune response using transferred to non-immune 14 day mice and lethally challenged to study the protection. Results indicated that dominant immune response of T helper (Th) 2 type. Th and neutralizing antibody inducing epitopes on JEV were identified by combination of immunological and Bioinformatics platforms. Chimeric peptides incorporating both Th and B cell epitopes could protect mice. These epitopes were further incorporated in polytope DNA construct with four chimeric peptides and induce protective immunity in mice. In addition, overcome the anergy development by traditional DNA vaccine plasmid than of CMV promoter using antigen specific cell promoter rather was also studied.

NIV carried out extensive studies on JE inactivated vaccines over the years. Studies were carried out mainly using CEC and Vero cells. Isolated of JEV from Kolar (821564) was extensively studied and thermostable mutant (821564 –XY) was selected and characterized genetically as well as antigenically. A commercial successfully produced purified, inactivated vaccine JENVAC is licensed is being successfully. Future challenges in terms of single dose vaccine with long lasting immunity, pig immunization vaccine as well newer related flavivirus are also important.

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DR. PRAN NATH CHHUTTANI ORATION delivered during NAMSCON 2015 at the All-India Institute of Medical Sciences, Patna.

INTRODUCTION

An infection in animal has mediated trying to multiplication and growth and thus will have host- virus alternatives. A virus through entering mosquito to peripheral skin levels in Langerhans cells and peripheral antigen presenting cells (APCs) can understand large alternative approaches. Thus just inactivating cells, multiplication and cytokines and interferon levels to decrease, can increasing high levels of cells to pass through blood brain barrier junction or restriction and further in neuronal cells to damage further pathogenic even upto deceasing (1).

A Japanese encephalitis virus (JEV) is a vector-borne viral disease that occurs in South Asia, Southeast Asia, East Asia, and the Pacific. The disease can cause irreversible neurologic damage. The JE virus (JEV) is mainly transmitted by the mosquito *Culex tritaeniorrhynchus*, which prefers to breed in irrigated rice paddies. Wading ardeid water birds serve as virus reservoirs, but the virus regularly spills over into pigs, cattle and humans. Because infected pigs act as amplifying hosts, domestic pig rearing is an important risk factor in the transmission to humans. JE prevalence has been shown in these animals mainly by isolations and also by seroconversion. In India also, many studies have shown the dominance of *Culex tritaeniorrhynchus* as a major vector (2, 3).

Central nervous system (CNS) infections due to its preferred position and protection are rare, however, when any CNS abnormality happens it manifests as a very severe disease. In addition, residual damage due to loss of nervous tissue also leads to disabilities at various physical and mental levels. There are numerous medical conditions which may produce an encephalopathic illness; from traumatic to metabolic to immunopathological causes, which may mimic viral encephalitis. Encephalitis refers to an acute, usually diffuse, inflammatory process affecting the brain. While meningitis is primarily an infection of the meninges, a combined meningoencephalitis may also occur. An infection by a virus is the most common and important cause of encephalitis, although other organisms may sometimes cause an encephalitis. The time course of the viral encephalitis can be acute, subacute, or chronic. Pathologically there are encephalitis with direct viral entry into the CNS in which brain parenchyma exhibits neuronal damaging and viral antigens (4). The work of National Institute of Virology and our group in Immunology Division is being submitted as a comprehensive in the form of review. Brief in studies of basic peptide development, immunopathology studies, DNA vaccine have studied. Development of inactivated, tissue culture based, Indian based JEV vaccine in collaboration of NIV and private commercial agency has been launched since 2014.

Immunopathological studies :

Innate immunity studies:

During an infection, interplay between the host immune response and the virus like virulence factors, capacity to evade the host immune response, play an essential role in influencing the disease outcome. Innate immune response represents the first line of defense, and it is triggered at the first instance of pathogen exposure by peripheral antigen presenting cells (APCs) and innate lymphocytes - $CD56^+$ natural killer/natural killer T (NK/NKT) cells (5). On viral infection, these cells undergo maturation and activate a cascade of anti-viral immune responses as well as act as the scaffold for establishment of adaptive response. Macrophages and DCs are professional APCs, which on antigenic stimulation are functionally activated resulting in induction of surface co-stimulatory molecules, and cytokine and chemokine responses that are required for establishment of effective anti-viral response. Studies have also shown that viruses are capable of evading/ modulating the host immune response, depending on their virulence factors. Primary innate anti-viral response is established due to action of soluble mediators such as - type-I interferon (IFN), nitric-oxide (NO); and functional activation of APCs and $CD56^+$ (NK/NKT) cells.

Two JEV strains JE057434 pathogenic, and SA14-14-2 were used in the study to understand the effect of viral virulence on the functional status of primary human monocytes derived macrophages (MDMs) and dendritic cells (MDDCs). Virus growth kinetic studies showed that MDMs support replication of both pathogenic and vaccine. Pathogenic JEV induced relatively lesser TNFα as compared to vaccine strain. The ability of JEV to induce type-I IFN from MDMs was dependent on virus dose and viral replication, rather than viral virulence. Transcript levels of OASs and RNaseL increased but remained identical between two strains at 18 hpi and 24 hpi in MDMs. However, Mx transcript increased in vaccine infected MDMs than pathogenic strain suggesting differential sensitivity towards Type-I IFN. Also, in order to determine whether pathogenic JEV infection mediates STAT1 degradation as compared to vaccine JEV, total STAT1 levels in JEV infected MDMs were determined at various time-points (i.e. 12, 24 and 48 hpi).

At lower IFNα concentration (10 IU/ml), IFNα pre-treatment resulted in complete abrogation of vaccine virus levels until later time points, however, in pathogenic JEV it was observed only for 24 hrs which it recuperated subsequently. Based on these observations, it could be concluded that JEV strains are susceptible to anti-viral effects of IFNα, though the sensitivity varies. The vaccine strain is more sensitive to pre-established anti-viral state than majority of the wild-type strains used in the study (6).

Interaction between DC and innate lymphocytes (i.e. $CD56^+$ cells) represents a crucial event during anti-viral innate immune response. Apart from macrophages, DCs are known to support flavivirus replication. During viral infections, NK and NKT ($CD56^+$ cells) are known to contribute to anti-viral response and aid in DC maturation. Presence of IL2-activated $CD56^+$ cells enhanced im-MDDCs maturation. Conversely, resting $CD56^+$ cells were unable to modulate CD86 levels and most of the cytokine or chemokine tested, with an exception of IL6. Co-culturing of resting $CD56^+$ cells led to an increased IL6 levels in un-infected and vaccine virus-infected im-MDDCs cultures. It was also seen that IL2-activated $CD56^+$ cells induced im-MDDCs maturation partially through direct cell-to-cell contact and TNFα, as separation of the cells by a permeable membrane or presence of neutralizing antibody against TNFα abrogated DC maturation. The IL2-activated $CD56^+$ cells showed higher degranulation (CD107a) capacity resulting in increased DC killing and greater ability to reduce JE viral load than resting $CD56^+$ cells (7).

In conclusion, the data indicates that JEV interacts with MDMs/im-MDDCs at different levels and would depend on both viral and host factors. The viral virulence might be related with replication fitness and a decreased susceptibility to primary anti-viral response. Therefore, reduction in infectious virion production and increased sensitivity towards primary anti-viral response of vaccine JEV strain could facilitate efficient virus clearance and limiting viral spread into additional target tissues and thus aid in mounting up of beneficial immune response.

Immunoprotective adaptive response:

Once the virus is grown in cells and is destroyed from the cells adaptive immune response starts the mechanisms. Basic start rules are, APCs cells they took presenting killed or live virion on the MHC type I and II antigens. Correlation with MHCs with the presenting to peptide to CD 4 and CD8 cells work the lymphnode cells. Activated lymphocytes T cells and B cells multiply and destroyed various CTL cells, antibody productions in form of IgM and IgG antibodies and cleared out the infected virions. Neutralizing antibodies of specific types are generated in blood and brains and make the cells long term protection by viruses.

In order understanding in model mice virus and lymphocytes in details are studied. Adoptive transfer studies were carried out by generating JEV immune splenocytes in adult BALB/c mice and transferring these primed effector cells into naïve 14-day-old recipient animals for survival analysis following lethal JEV infection. The contribution of CD4+ or CD8+ T cell subsets to protection was also examined by either depleting or isolating these subsets from JEV immune splenocytes before adoptive transfer and determining the survival rate in recipient mice. In addition, the nature of the T helper response in adoptively transferred mice was studied by analyzing the cytokine profile and antibody subtypes

produced in response to JEV infection. Differential gene expression profiling of brains from adoptively transferred mice was also performed in order to identify critical genes responsible for the modulation of infection at the target site (8).

The adoptive transfer of JEV immune splenocytes into naïve 14-day-old recipient mice resulted in the protection of 95% of the recipients from peripheral JEV challenge. The survival rate was reduced when transferred cells were depleted of the CD4+ T cell population 34.62% survival. Correspondingly, increased protection was observed when JEV primed CD4+ isolated T cells were transferred, as compared to CD8+ isolated T cells 53.85% and 28.57% survival respectively. Concurrent with results indicating a definite role for CD4+ T cells in protection from JEV infection, it was seen that the repeated in vitro stimulation of JEV immune splenocytes with a peptide representing a T helper epitope from the prM region of JEV, followed by adoptive transfer of these cells into naïve mice was capable of conferring immunity in these animals from subsequent lethal JEV challenge. In addition, transfer of splenocytes from peptide immunized animals was also capable of protecting naïve recipient mice from infection with JEV (9).

Real time PCR analysis of virus titres in the organs of mice receiving JEV immune splenocytes revealed similar kinetics of virus replication at peripheral sites as in lethally infected mice. Virus titres in the brains were, however, much lower in mice that received JEV immune cell transfer. This suggested that mortality from JEV infection was in some part due to virus load in the CNS of infected mice and associated immunopathology. Mice receiving JEV immune splenocytes had considerably reduced levels of IFN-γ and TNF-α in the sera. In contrast, sustained expression of the Th2 cytokines, IL-4 and IL-5, was observed at all-time points post infection. Differential gene expression studies from the brains of mice that were protected from JEV infection revealed a considerable increase in the expression of immunomodulatory cytokines like IL-4, IL-10 and TGFβ.

In conclusion, protection from lethal JEV infection in naïve 14-day-old mice involved a CD4+ T cell mediated, Th2 immune response. In mice receiving JEV immune splenocytes, faster kinetics of Th2 antibody production resulted in higher levels of JEV specific antibody in the sera, which probably helped to reduce virus load in the CNS. Reduced levels of proinflammatory cytokines like IFN-γ and TNF-α in the sera of protected mice, combined with an increase in Th2 cytokines like IL-4 and IL-5 probably achieved an immunomodulatory effect that resulted in the enhanced survival of these animals.

Vero cell derived inactivated Indian JE vaccine :

Vaccination is the single most important measure to control Japanese encephalitis. It is recommended that JE

immunization of children should be continued according to established schedules in regions where this vaccine has already been successfully introduced, preferably as part of the national immunization programs. NIV has carried out extensive studies on JE inactivated vaccines over the years. Studies were carried out mainly using CEC and Vero cells. The unpurified formalin inactivated vaccine has been studied. The blood sample from encephalitic patient (Lx-9 yr. female) was collected in 1981 (Nov-Dec) and was processed for isolation of etiological agent. Which was further confirmed as Japanese encephalitis virus using complement fixation test and neutralization test. This was named as 821564. From this parent strain of JEV (821564-XZ) thermostable mutant (821564 –XY) was selected and characterized genetically as well as antigenically. This thermostable mutant was analyzed further for vaccine development. Antibody response against various strains in India and HI titres of mouse serum prepared against different JEV strains was studied. Shelf life of the vaccine using thermostable strain of 821564 XY was found better than the parent JEV 821564 XZ strain.

Development of Thermostable Mutant of JEV-821564XY lyophilized M.Br.Susp was under MOU was transferred to Bharat Biotech International Ltd for Vero cell adaptation and vaccine development. The strain got readily adapted to Vero cell and BBIL then successfully produced inactivated vaccine. The antigenic as well as genomic differences between the two variants of 821564 were analyzed using different techniques. BBIL produced Vero cell inactivated JE vaccine. Purified and inactivated virus vaccine that has passed all the production quality testing would be used at desired concentrations. Suspension of vaccine preparation in aluminum hydroxide adjuvant would be carried out as per the standard procedures of manufacturing. The approved dose of E protein content in JENVAC is 5 µg /dose. The vaccine subjected to various regulatory tests and trials. The vaccine JENVAC is about to be licensed for use in humans.This patent application also covers another aspect, at specified concentrations, are added to stabilize the purified live Japanese encephalitis virus bulk during inactivation process simultaneously (10).

Integrated studies at NIV leading to vaccine development :

Generation of mouse monoclonal antibodies (MAbs) were generated and carried for envelope proteins. These originated escape mutants classifications of JEV and flaviviruses were analyzed. IgM ELISA diagnosed was developed for JEV and DEN assays. These were supplied all over India in Govt. Health Systems since more than 7000 kits each year. There are MAbs derived JEV detection methods have also been worked.

The project was a continuation of collaborative efforts between Bio-informatics Centre, Department of Biotechnology Pune University and

National Institute of Virology was studied as basic studies. While 3D envelope *in silico* and we could develop various peptides that could reacted with MAbs. This related B epitopes by synthesis peptides that were JEV neutralizing peptides.

As a part of Bioinformatics sciences of primary proteins again *in silico* in analysis T helper epitopes for Envelope and NS-1 and NS-3 of JEV was synthesized. Using immune splenocytes we could analysis good T helper peptides also. As both combining T helper and B cells peptides called - chimeric T helper B cells epitopes were synthesized, immunized in mice and could lethal challenge. Single chimeric peptide could protect mice protection also (11-13).

During these studies DNA vaccine was known to use for envelope etc. We also using combinations of four chimeric epitopes together in DNA plasmids. Therefore, it became imperative to design a construct containing multiple epitopes (polytope) as it would be a superior vaccine candidate for further enhancement of the immune response. Adjacent epitope units in the polytope construct were joined by highly flexible spacer sequences as linker, Polytope construct of JEV (P-JEV), was cloned in-frame into pcDNA3.1/V5-His which is a transient expression vector(14). The protection observed following challenge of mice immunized by intramuscular route was 70% (15,16).

DNA vaccine offers an alternative strategy which can be further improved upon by incorporating various adjuvants. Successful immune response requires engagement of T cell receptor with MHC-peptide on professional APC as first signal. Simultaneously, second signal in the form of various co-stimulatory molecule engagements is necessary for sustained immune response. Failure to have this second signal may lead to reduced immune response or even anergy. Targeting DNA vaccine to APC has been studied. It can be argued that widespread expression of a gene through viral CMV promoter may lead to some adverse effect. Our attempt for immunomodulation was to target the antigen expression in professional APC by using the selective promoters. This could prevent anergy by expressing the antigen dominantly in professional APC. The constructs were further manipulated with the partial C, prM and E (E) genes of JEV encoded by promoters of macrosialin (pMS-E), CMV (pCMV-E) and promoterless constructs (pNIX-E). Neutralizing antibody response to JEV showed that pCMV-E induced 1:450 titer while pMS-E induced 1:300, control plasmid and PBS did not show any detectable N'Ab titers. In summary, we have demonstrated herein the *ex vivo* expression of E protein directed by CMV and macrophage active promoter (16, 17).

Future vaccine projects :

Many newer developments using as basic studied already available can be used. In order to develop long term neutralized inactivated can be used. This means further using JENVAC inactivated

by adding T cells or chimeric peptides. Some basics are known results already present.

In order to develop DNA vaccines envelope along with chimeric epitopes in form of DNA vaccine for pigs can be used. These will be low cost pigs available. This will also means that before seasons we can immunized JEV infected and detecting amplified vaccine in field can also be worked. This even might decrease mosquito-JEV also.

During last few years both in Assam and Kerala human West Niles are being circulated in seasons. There is a need to be studied newer vaccines for humans at the earlier. As a part of inactivated WNV inactivated are needed based on Indian WN current viruses. In addition some studies showed that simultaneously CTL are also needed. It is possible that inactivated and CTL-T helper chimeric peptides together need to be incorporated.

NIV Scientifics along with Contributors and Collaborates over last about 60 years has some of the based inputs. I hope we start various things for our on Indian studies.

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Ann Natl Acad Med Sci (India), **52(2):** 109-123, 2016

Modern concept of Benign Breast Disorders and its Endocrinological Background

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ABSTRACT

Mastalgia and generalised breast nodularity where a discrete lump cannot be felt called **Aberration of Normal Development and Involution** (ANDI) is the most common condition leading to breast related consultations. The author's work on this clinical syndrome over the last 3 decades has led to documentation of its natural history, epidemiological study of ANDI, development of objective scales for clinical assessment of breast pain and nodularity, its endocrinological aetiopathogenesis, response prediction of treatment in cyclical mastalgia, a randomized controlled trial of an indigenous selective estrogen response modulator – ormeloxifene and finally a meta-analysis of treatment for this condition. This turned a full wheel bridging gaps in the knowledge of this disorder and led us to evolve an effective, inexpensive treatment of mastalgia with least side effect. The work is dedicated to Professors LE Hughes, Robert E Mansel and Anurag Srivastava.

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COL. SANGHAM LAL MEMORIAL ORATION delivered during NAMSCON 2015 at the All-India Institute of Medical Sciences, Patna.

INTRODUCTION

Nine out of ten breast related consultation consists of benign breast disorders that present as pain, inflammation, nipple areola problems, discrete lump and nodularity are common. The epidemiology of benign breast disease was not much studied. With social, economical, educational and information evolution increasing number of women solicit medical opinion for simple pain and nodularity in breasts. There is a considerable morbidity amongst women in India on account of breast pain and nodularity. It is indeed a normal finding and a physiological aberration of normal development and involution. This fact is still not very well understood by the general practitioners, gynaecologists and surgeons involved in the care of breast disease. Unnecessary biopsies and cancer phobia is commonly generated on account of simple benign breast nodularity.

Breasts are composed of epithelial system of ducts and lobulo-alveolar secretary units embedded in adipose tissue with interspersed fibrous septae derived from mesenchymal tissue. Morpho-genesis of these tissues occurs with hormonal changes, genetic constitution and mutation that respond to the circulating hormones and milieu interior. Paracrine effects of locally derived factors affecting the epithelium and stromal relationship form the major basis of benign breast disorders mainly breast pain and nodularity. Histo-pathogenesis of painful nodularity of breasts was erroneously described (1-3). The natural history in a large cohort of such subjects was described by the author (4). Two populations emerged; cyclical pronounced mastalgia (CPM) and non-cyclical mastalgia (NCM). The cyclical pain generally related to a hormonal event, started early in life and usually abated at menopause spontaneously. This condition was described as **ANDI or Aberration of Normal Development and Involution**. It has no histological basis except for hormonally mediated change characterized by lumpiness of the breast and varying degrees of pain and tenderness. Retrospective epidemio-logical studies from the hospital data showed that fibroadenoma was the commonest benign breast lesion to be operated (5). The prevalence of nodular breast in the community (10%) and hospital based population (20%) was first described by the author using a validated 5-point objective assessment of breast nodularity scale (6). The assessment of mastalgia requires a careful structured history and physical examination and assessment of at least 2 months filling of pain charts to determine whether it is cyclical pronounced mastalgia (CPM) or non-cyclical mastalgia (NCM). In a quarter of patients it may be difficult to clearly distinguish between the two patterns and empirical management could be adopted.

Cyclical Pronounced Mastalgia (CPM):

The commonest pattern seen in about two third of the patients is cyclical pronounced pattern because of its

temporal relationship with menstrual cycle. It is almost always premenstrual with a duration varying from 1 – 4 weeks. Many women experience 2 – 3 days premenstrual breast tenderness or heaviness and this should be regarded as normal. CPM almost invariably has nodularity of a varying degree associated with it. This nodularity is maximal in the upper outer quadrant and shows cyclical changes. Fine nodularity that begins a short time before menstruation and regresses postmenstrually should be regarded as normal. The symptoms are maximally seen in the 3rd and 4th decades of life. However, cyclical breast pain is also encountered in young girls, women who have had hysterectomies and in peri menopausal women. CPM is regarded significant clinically if its intensity is 'pronounced'. The pronounced cyclical mastalgia is defined either by duration of greater than one week per cycle or by severity using a pain chart. Severity of pain is indeed, like all assessment of pain in clinical practice, a subjective assessment. Obstructive features in life style, such as sleep loss, work disturbance, breast tenderness or interrupted sexual activities are additional pointers towards the diagnosis of pronounced cyclical mastalgia. Other characteristics of CPM are bilateral pain associated with nodularity, heaviness and tenderness to touch. The pain often radiates to axilla and down the medial aspect of the upper arm. Clinical examination reveals varying grades of tenderness and nodularity especially in the upper quadrant. Nodularity has to be differentiated from a discrete lump felt with the flat of the hand. Occasionally there may be small mobile nodes in the axillae. Examination in the premenstrual period often reveals greater degree of tenderness and nodularity when compared to the examination in the post menstrual period of the same patient. A discrete lump can be associated with pronounced cyclical mastalgia and qualifies for independent assessment. However, specific attention should be given to thyroid examination, gynaecological examination and other endocrinal manifestation because of some quasi association of CPM in such conditions.

Careful history, physical examination and pain charts are usually enough in the work up of mastalgia patients. Detailed endocrinological investigation is warranted only in a research setting or in the presence of overt endocrinal features. Mammography has proven unhelpful in the assessment of CPM as the non-specific changes ascribed to fibroadenosis have so far been the main feature. No specific radiological appearance correlates with the site or side of pain. Mammography is often ordered by the physician to relieve anxiety and forge reassurance. Such use of mammography is unwarranted and can lead to unnecessary demand on the services and un-indicated mammography in women below 35 years of age who frequent for such a problem. Selective screening of patients with symptomatic breast has no scientific basis as mastalgia is not a known risk factor for breast cancer and use of mammography in this situation is not cost effective. The role of FNAC in

the assessment of CPM patients is limited only to associated discrete lumps.

Non-Cyclical Mastalgia (NCM) :

A third of the patients with breast pain on detailed assessment of their clinical features and pain chart are distinguished principally by its lack of relationship with the menstrual cycle. This non-cyclical pain pattern is observed in both pre- and postmenopausal women. The NCM pattern differs in several other aspects from the cyclical. The pain tends to be well localised in the breast and is more frequently sub areolar or inner quadrant in location. A finger-pointing test may be present. Simultaneous and similar bilateral pain is uncommon and descriptive terms of burning, drawing or abscess like are used by the subject. Transient sharp, pricking or stabbing pain may be experienced by some subjects suffering from NCM. When assessed on the linear analogue scale, the non-cyclical pattern is scored by the patient at a lower intensity than the cyclical pattern. Physical examination may reveal discrete areas of tenderness within the breast that may demonstrate, 'trigger spot pain', implying continued complaint of pain after palpation of those areas. Nodularity is less prominent than in the cyclical group and there may be no palpable abnormality at the site of pain. The pain is reported maximally in the fourth decade of life. A minority of these patients may actually be having cyclical mastalgia and represent the overlap that can occur despite the evaluation of pain charts. The natural history study undertaken by the author revealed that if untreated, non-cyclical mastalgia showed spontaneous remission in half the patients unrelated to hormonal events in the body. There were two groups, the first in which the pain lasted for few years and the other in which it continued for over twenty years. Endocrinological investigations are unhelpful and unnecessary. Mammography has been of some interest in this group of patients. Radiological changes of coarse calcification and ductal dilatation attributed to ductal ectasia or periductal mastitis have been commonly seen in this group. The role of FNAC is limited. A variety of distinct histological features may be associated with NCM. These include duct ectasia, or periductal mastitis, sclerosing adenosis, trauma, post biopsy, and miscellaneous breast lesions. A minority of patients with carcinoma breast may present with NCM. However, in approximately half the patients with NCM there may be no underlying histological change demonstrable.

Aetiology of Benign Breast Disorder :

As for aetiology of mastalgia and nodularity, in the past, benign breast disease for most doctors it has been regarded as synonymous with "fibroadenosis" or "fibrocystic disease". These terms were used for the syndrome of premenstrual pain and nodularity. This concept arose from the unfortunate fact that early workers described histological changes of fibrosis, adenosis, cyst formation and apocrine metaplasia and assumed a causative association(1). Subsequently it was well established that

these histological changes were normal features of the breast microanatomy and ubiquitous in nature. As such there are no histopathological changes ascribable to breast pain and nodularity described so far. After extensive studies and review of literature, the major concern towards the risk of subsequent cancer or the pre-malignant potential of fibrocystic disease was set aside (2). This led the authors to put forward the concept that fibrocystic disease was a non-disease i.e. it did not exist. Long term follow up of specific benign lesions on histology for their subsequent cancer risk were clearly described and showed no association with mastalgia (3). This concept is attractive in denying the supposed histological basis for the clinical condition of mastalgia. However, the problem of providing satisfaction to many women who suffer a variety of clinical symptoms of distressing severity remains. The morbidity both physical and psychological on account of nodularity and breast pain needs to be addressed. The present consensus is to regard mastalgia as a purely clinical symptom complex, which should be included as a part of a broad based nomenclature suggested by Hughes *et al* (1989) (3), "ANDI or Aberration of Normal Development and Involution". It is based on the fact that most benign breast disorders are relatively minor aberrations of normal process of development, cyclical hormonal response and involution that interact throughout a woman's life. The term "aberration" has been included because it also encompasses a spectrum from minor to marked changes. It covers the common, major benign processes of the breast like pain and nodularity, duct ectasia or epithelial hyperplasias. The concept of ANDI can be extended to cover a full spectrum of severity - from normal variations to disease. It is simple and consistent with the current knowledge of aetiopathogenesis.

In the past decade systematic investigations have been undertaken to understand the aetiological basis of mastalgia. Astley Cooper started the trend of describing the mastalgia patient as being of nervous disposition. However, case control studies undertaken using validated psychoneurotic score questionnaire did not confirm this view and treatment trials with anti anxiety drugs were unsuccessful. Secondly, water retention or oedema as aetiological basis of mastalgia when investigated scientifically was not confirmed. There is no report in the literature suggesting that general oedema was associated with mastalgia. Therefore, there is no rational basis of treatment of mastalgia with diuretics. The beneficial effect seen in general practice from diuretic treatment is due to placebo effect. Thirdly and perhaps most appropriately earlier workers who did not have detailed knowledge of hormonal profiles have suggested a hormonal basis for mastalgia. The advent of accurate radioimmuno-assays for estimating the blood hormones resulted in a large number of studies that tried to look into the issue. Three main theories have thus emerged regarding the aetiology of painful nodular breasts:

1. Increased oestrogen secretion from the ovary.
2. Deficient progesterone production (or 'relative hyper-oestrogenism')
3. Hyper-prolactinaemia.

Several studies have been performed on the above three theories. The results for CPM are difficult to interpret because of the too often mixing of the pathological and clinical terms. The balance of evidence, however, suggests that the first two theories are unimportant as the steroids levels were no different in clinically well-defined patients and controls. The case of luteal defect although strongly supported by a French group was not seen in other studies. Random levels of prolactin showed no significant difference between patients with benign breast disorders and controls. A major problem here is that prolactin secretion in normal women is pulsatile and has diurnal variation. Therefore, random sampling of basal prolactin is inappropriate. Careful studies of daily sampling at a fixed time throughout the menstrual cycle reveal a small but statistically significant difference between women with breast disease and controls. Prolactin is secreted by the anterior pituitary and is tonically inhibited by dopamine secretion by the hypothalamus. However, prolactin secretion by the pituitary can be stimulated by the use of thyrotropin releasing hormone and dopamine antagonist agents like Metoclopramide and Domperidone.

Several other aetiological theories have been proposed. The over stimulation of breast cells due to interference with ATP degradation by methylxanthine has some biochemical evidence to support it. Excessive coffee intake, which is a rich source of methylxanthine, has been incriminated by one group in North America. Caffeine intake in Indian women with mastalgia is much lower and may not be relevant in India. Another hypothesis proposes an abnormality of prostaglandin synthesis due to deficient intake of essential fatty acids (EFA) in diet. The result of EFA deficiency, however, may be a representation of the amplification of prolactin effect on breast cells because of deficient production of prostaglandin E1.

Author's Contributions :

1. Natural History of Mastalgia :

In order to document the natural history of mastalgia (4) in untreated subjects 258 patients with breast pain were re-studied 2 to 7 years after initial assessment in a special mastalgia clinic. Pain persisted at follow up in 65% of patients. Mastalgia was cyclical in 2/3rd mean duration of pain in patient experiencing complete relief before follow up examination was 6.8 years, while duration of pain persisting at follow up ranged from 2 to 30 years. In patients who had relief or substantial improvement in pain, the improvement was spontaneous in 22% and resulted from a hormonally related – menopause, pregnancy, or use of oral contraceptives –

in the remainder. Onset of cyclical pain before the age of 20 years was followed by a prolonged course. A quarter of the patient had non-cyclical pain. There were 2 population of patient in this group. One experienced relief after a mean of 3 years, and in the other pain still persisted after 2-20 years. Relief was spontaneous in one half, and rarely followed a hormonally related event. About 70% of the patients, with both cyclical and non-cyclical pain, considered that there pain had warranted active treatment. This study indicates that the type of pain and age at onset may allow some prediction of the course of the disease and may aid the choice of therapy.

2. The Epidemiology (5) of Benign Breast Disorders (BBD) :

BBD was studied by the author both in the hospital and community. Experience with BBD has been analysed in 3 non-western populations: Hong Kong, India and Northern Nigeria. Similarities to and differences from Western experience are found, but of great interest are notable differences between these populations which, as yet, lack explanation. All show 'fibroadenosis' and fibroadenoma as common conditions, but the frequency with which phyllodes tumor is diagnosed varies between centers in India as well as between different racial groups. Tuberculosis is another interesting example – wide differences in the frequency in all 3 countries. The value of prospective studies was shown when mastalgia was studied in this way in India. Often considered a 'Western' affliction, we were able to study 112 cases of mastalgia and found it to be at least twice as common as cancer as a presentation in hospital based practice in 1970s and 1980s. These differing experiences between populations have yet not been explored and must hold promise for unraveling some of the enigmas of benign breast disorders in all countries (5). Furthermore, in 2010 the author studied 784 Women (hospital 384; community 400) aged between 20 and 70 years (mean 31.9) who underwent physical breast examination by 2 experienced clinicians. Inter-observer matched nodularity grading in women attending hospital were Grade 0 in 123 (32.03%), grade 1 in 67 (17.44%), grade 2 in 54 (14.06%), grade 3 in 52 (13.54%) and grade 4 in 23 (5.99%) and in community it was grade 0 in 172 (43%), grade 1 in 88 (22%), grade 2 in 60 (15%), grade 3 in 28 (7%) and grade 4 in 14 (3.5%) women. There was very good agreement (kappa ¼ 0.7798) across all grades in hospital subjects and excellent agreement (kappa¼ 0.8659) in community subjects. Both estimates of kappa coefficients were highly significant from population kappa coefficient of zero ($p<0.001$). Overall, 1/3rd normal women have absolutely smooth textured breasts (6).

3. Lucknow Cardiff Breast Nodularity Scale :

In the above study a **scale for clinical assessment of nodularity** (6) was also developed for the first time and published. Objective measurement of benign non-discrete lumpy breasts is not performed routinely that would lead to

disease measurement, inter-physician communication, therapeutic response assessment and a normative function of reducing unnecessary biopsies. A schematic 5-point ordinal visual analogue scale was conceptualised. Two blinded experienced clinicians graded breast nodularity on a pre-determined five point analogue scale (grades 0–4) to determine its inter-observer reliability after its face validity that excluded inflammatory, nipple, areola and discrete lump problems. User-friendly tool developed for objective evaluation of non-discrete lumpy breasts showed excellent reliability and validity. This tool should be useful for clinical drug trials in benign breast disorders and for wide routine clinical recording of patients.

4. Endocrinological Background and Aetiology of Benign Breast Disorders :

It was generally known that there was no overt alteration in the circulating levels of various peptide and steroidal hormones of adrenal and gonadal origin in benign breast disorders. The pituitary control of prolactin secretion by TRH stimulation and domperidone dis-inhibition in well-defined CPM patients and controls were examined. Several blood samples were collected at regular intervals from these subjects and careful radioimmunoassays for prolactin were carried out. These studies have shown that basal prolactin levels were not significantly different between the groups but stimulated prolactin response and peak prolactin release was significantly greater in CPM as opposed to NCM patients and controls. Similar results have been reported by an earlier worker from Germany. These data strongly suggest that a functional or a subtle 'fine tuning' defect may be the primary problem in painful nodular breast disease. Interestingly, a similar defect has been demonstrated in the cyclical oedema syndrome that has many similarities to cyclical mastalgia although they are distinct conditions. The excitability of prolactin secretion provides the basis of successful treatment of cyclical mastalgia using the dopamine agonistic agent - Bromocriptine. The effectiveness of this drug has been shown in several controlled clinical trials (7).

Paraffin wax embedded formalin-fixed BBD tissue taken from 17 patients (15 with microcystic disease and 2 with fibroadenoma) was studied for the presence of tissue bound prolactin using a rabbit antiserum against human prolactin applied in conjunction with a highly sensitive modified version of the dinitrophenyl (DNP)-hapten sandwich staining (DHSS) procedure. Sections taken from 14 of 15 cases showing apocrine cystic changes exhibited strong prolactin staining restricted to the cytoplasm of metaplastic apocrine cells lining the cyst. Normal lobules and ducts and blunt duct proliferations were all negative, as were also the 2 cases of fibroadenoma. In contrast 6 out of 8 cases of breast cancer examined showed heterogeneously distributed cytoplasmic staining in the cancer cells. Maximal prolactin staining in the apocrine cells was observed at antiserum dilutions as high as 1:60,000. This compared favourably with

a 1:120,000 dilution that gave maximal levels of staining in the prolactotrophs present in serial sections taken from formalin fixed paraffin wax embedded post mortem human anterior pituitaries. In both types of tissues the specific staining was abolished by pre-absorption of the antiserum with human prolactin (10 micro gram ml-1). No staining was observed when the anti-prolactin serum was either omitted or substituted with DNP-labelled normal rabbit serum. Apocrine metaplasia in cystic disease of the breast has been found to be associated with an increased breast cancer risk. The strong and selective presence of immunohisto-chemically demonstrable prolactin in the metaplastic cells may be of significance in view of the hormone's known growth stimulating effect on the breast epithelium (8).

Hypothalamic pituitary axis tests and prolactin (9-11) :

Pituitary function was tested in predefined clinical groups of benign breast disease under strictly controlled clinical and laboratory conditions. Two different tests of prolactin storage and control mechanisms, direct stimulation by thyrotropin-releasing hormone (TRH) and inhibition of dopaminergic control by domperidone, indicate a significantly abnormality in patients with severe cyclical mastalgia and nodular breast disease ($P<0.05$ and $P<0.002$), but not in those with noncyclical mastalgia. No abnormalities of thyroid function were found (9). Furthermore, a generalized abnormality of hypothalamopitutary function was found in 17 patients with cyclical pronounced mastalgia compared with 11 controls by using a combined thyrotrophin releasing hormone and gonadotrophin releasing hormone test. The release of prolactin, luteinizing hormone and follicle stimulating hormone was significantly greater in cyclical mastalgia patients than in controls. Basal thyrotrophin, T3 and T4 levels were within the normal range in both groups indicating normal thyroid status in benign breast disease. The single measurement of oestrogen and progesterone in the luteal phase was not abnormal. These data demonstrate an alteration in lactotroph and gonadotroph function in patients with cyclical mastagia. It is unknown at present whether this represents an appropriate cellular response to altered central or peripheral signals. There is no evidence to suggest, however, that the anterior pituitary cell types are abnormal per se (10, 11).

Corpus luteal function test – daily salivary progesterone (12) :

Progesterone levels were measured in samples of saliva collected daily throughout the menstrual cycle in patients with pronounced cyclical mastalgia and breast nodularity. A control group matched for age, length of menstrual cycle and parity was also studied. No significant differences in progesterone levels were detected between the two groups for the luteal phase of cycle. These data indicate that cyclical mastalgia is not associated with significant luteal phase progesterone

insufficiency, as demonstrated by salivary levels and, by implication, serum levels of progesterone.

5. Prediction of Response to Endocrine Therapy in Mastalgia (13) :

Many of the endocrine agents currently used to treat symptomatic BBD modify the action or secretion of prolactin. We have compared the responses to hormonal therapy with dynamic assessment of prolactin control in 29 patients with CM and 9 patients with NCM. The tests of prolactin release used were direct stimulation with TRH or dopaminergic blockade by domperidone carried out before treatment in mastalgia patients and 22 age–matched asymptomatic controls. The response to treatment was assessed using a special pain chart and visual linear analogue scale. Patients with cyclical mastalgia could be divided into two groups: those in whom the peak prolactin release was exaggerated (mU/l) and those in whom the prolactin release was less marked and similar to control subjects and patients with non-cyclical mastalgia. Patients in the cyclical mastalgia group with a high peak prolactin release responded to hormonal treatment significantly more frequently (90%) than those with a normal prolactin release (50%). Basal prolactin levels did not correlate with the response to treatment. In the non-cyclical mastalgia group, no patient had peak prolactin release was exaggerated (greater than 4000 mU/l) and none responded to therapy. This study indicates that dynamic tests of prolactin release in cyclical mastalgia may be useful in predicting the subsequent satisfactory response to endocrine therapy if a high peak prolactin release is induced.

6. Benign Breast Tissue Characterization :

Prolactin receptors :

In another study (14) an immunocytochemical method involving the application of polyvlonal antisera to human prolactin (PRL) followed by a highly sensitive and a modified version of dinitrophenyl (DNP) hapten sandwich staining procedure using anti-DNP IgM monoclonal antibody has been used to detect PRL binding in benign and malignant breast tissue. The technique was applied to 5 microns thick sections of paraffin embedded formalin fixed tissue. Out of 107 breast biopsies 40 were carcinomas, 41 were fibroadenomas, 18 were benign cystic disease and 8 were gynaecomastia. In cases of carcinoma positive staining was observed in 82.5% whereas in fibroadenoma the positivity was in 57% cases only. The positive reaction in fibroadenoma was mainly due to the presence of apocrine metaplasia associated with the tumor. Also PRL was present in greater proportion in postmenopausal patients as compared to premenopausal cancer patients. These findings suggest the presence of specific PRL binding sites in breast tissue. The staining was restricted to epithelial cells and background staining of the stroma was minimally seen in these cases. Positively stained breast carcinoma may

represent an apocrine subset of the carcinoma.

Human Sodium Iodide Symporter (hNIS) (15) :

Human sodium iodide symporter (hNIS), responsible for the active transport of iodine is an integral plasma membrane glycoprotein present in the thyroid cells and extrathyroid tissues like breast and salivary glands. If its functional form is unequivocally shown in benign or malignant breast tissues, then it may serve as a basis for diagnosis and treatment using radioactive iodine. With an aim to analyze the hNIS expression in a distinct benign breast condition of fibroadenoma, biopsy proven fibroadenoma tissues, normal non-lactating breast tissue and biopsy proven infiltrating duct carcinoma tissues were examined for hNIS expression using immunohistochemistry. Out of 20 biopsy proven fibroadenoma tissues, 19 (95%) showed positivity for hNIS protein and only one was negative. Of these 10% were mildly positive, 50% cases were moderately positive and (35%) showed intense positivity. None of the control tissue obtained from reduction mammoplasty specimens or normal breast tissues samples (5 cms away from the tumor) were positive. hNIS was also intensely positive in 9 out of 10 (90%) infiltrating duct carcinoma tissues and moderately positive in one case. These preliminary results show that hNIS was present in high frequency as demonstrated by immunohistochemistry in fibro-adenoma breast.

7. Treatment of Painful Benign Breast Nodularity and Meta-analysis :

Pain breast nodularity is treated by a large number of agents. These include hormonal manipulation by Danazol, Bromocriptine, Tamoxifen and LH-RH analogue ZOLADEX. Non hormonal agents effective in mastalgia are Nonsteroidal anti-inflammatory gels, iodides, plant derivatives like evening primerose oil (EPO) and Vitus Agnus Castus and reflex therapy. There was a considerable debate about the choice of best agents for initial management of mastalgia. No meta-analysis was described to evaluate the most effective agent. A meta-analysis on published randomized trials of common agents used in the therapy was attempted (16).

Articles on randomized trials on treatment of mastalgia were searched using the electronic databases viz. Medline, Google Embase, textbooks of benign breast diseases and surgery. The search was performed using key words: mastalgia, mastodynia, breast pain, benign breast disease, therapy & treatment and was confined to articles published in the English language. The search was also restricted to randomised clinical trials where an active drug was compared with placebo or another drug along with the placebo. Trials without randomisation or without a placebo arm were excluded. This meta-analysis was done for 4 agents that are commonly used. The outcome of interest was reported as the mean pain score with the active drug and with placebo in some

studies while other studies have described the number of cases achieving clinical response (usually greater than 50% reduction in the mean pain score measured on a visual analogue scale or Cardiff breast pain scale). Hence the pooled estimates of standardised difference of mean pain score between active drug and placebo has been calculated. The point estimates and 95% confidence limits have been computed for Fixed effect models. The pooled risk ratio (RR) has been computed for studies reporting outcome as dichotomous data on a 2x2 table. The Meta-analysis has been performed on "REVMAN" Meta-analysis statistical package from Cochrane Collaboration.

The results showed heterogeneity for the trials on Bromocriptine, the Chisquare test of heterogeneity yielded a p= 0.26 indicating that the results of the 3 trials were not heterogeneous. For trials on Tamoxifen the test demonstrated no heterogeneity ; p= 0.47. For trials on EPO the heterogeneity test yielded a p= 0.53. Thus a fixed effect model was applied to all these trials. A summary recommendation from this meta analysis emerged as patients with pronounced cyclical mastalgia can be benefited by a number of agents. Danazol, Bromocriptine, Tamoxifen are all effective in ameliorating the breast pain. Since very few studies have compared more than one agent in a RCT, the best choice of drug is difficult to make from a statistical point of view. Both Danazol and Bromocriptine produce significant side effects, some of which are highly undesirable in young women (weight gain, hair growth, menstrual irregularities, gastrointestinal upset, nausea and vomiting) hence their use is currently declining. Since the relative risk of pain relief with Tamoxifen is 2.11, tamoxifen should be the drug of first choice. The low dosage regimen of 10 mg daily for 3 months is shown to be as effective as a higher dose 20 mg, hence it should be tried first for 3 months as the initial drug treatment of mastalgia. A search for newer agent specially SERMs was envisaged (16).

8. Randomised Control Trial with Ormeloxifene (17) – a SERM :

Double blind randomized placebo controlled clinical trial of oral centchroman 30 mg (ormeloxifene); a SERM or placebo twice a week for 3 months in women (20-50 yrs) with pronounced breast pain with or without lumpiness were recruited after excluding discrete benign lump or cancer. Serial assessments of pain on a visual analogue scale and nodularity grade on a 5-point ordinal Lucknow-Cardiff scale were done. A total of 151 patients were randomly allocated to two interventions using blocks of size four. Participants and physicians were blinded to randomization. Of the 151 patients, 121 (active=57, placebo=64) were available for efficacy analysis. The mean pain level showed a systematic downward trend over five visits ($F=105.23$, $p<0.0001$), that significantly reduced in active group compared to placebo ($F=18.66$, $p<0.0001$). The patterns of variation in pain over time for the individual groups

differ from the overall mean pattern for two groups and thus from one another ($F=44.43$, $p<0.0001$). Cumulative frequencies of breast nodularity grades during the successive visits showed significant improvement ($p=0.001$) compared to placebo at the end of third month. The effect of active drug persisted till the completion (6 months) of the treatment ($p < 0.001$). At the last visit, 91.2% subjects in active group had grade 2 or lower nodularity as compared to 65.7% in the placebo. Oligomenorrhea alone was reported in 12 subjects. Centchroman showed significant efficacy for treating breast pain and nodularity.

Conclusion :

Painful nodularity of female breasts is a common clinical presentation both in general and specialised practices. ANDI is now a distinctive clinical syndrome without any histopathological basis. Pronounced mastalgia causes morbidity and requires treatment. It is not a symptom of neurotic women. Reassurance against absence of cancer is the main stay of treatment. Increased cancer risk is unassociated in the absence dysplasia. It has two distinctive patterns of cyclical pronounced (CPM) and non-cyclical mastalgia (NCM). CPM is common during child bearing age. It is amenable to hormonal treatment and has an hormonal aetiopathogenesis. There are no overt changes in the circulating levels of hormones, however. A subtle hormonal abnormality both in the circulating levels and target tissue is possible.

A thorough history and physical examination of breast, ultrasound in younger subjects and x-ray mammography in women above 35 years should suffice the clinical work up in a usual case. It is further added by using a breast pain chart to document the intensity and the pattern of pain, its temporal relationship with menstrual cycle. Lucknow Cardiff breast nodularity scale developed above is a reliable scale on 5 points of 0 to 4 grades. Grade 3 to grade 4 nodularity is seen in the Indian community in about 10% normal subjects. Unnecessary biopsies must be avoided. Objective assessment of pain and nodularity will allow the clinicians to measure the response to treatment.

In summary, the hypothesis of neuroticism and water retention has not found support from experimental data. Extensive hormonal studies both as basal, month long salivary hormones and dynamic hypo-thalamic pituitary axis tests in CPM and NCM revealed normal basal levels of oestrogens, progesterons, thyroid hormones and prolactin. Pulsatile secretion of prolactin and / or gonadotropins are abnormal in painful nodular breast that provided a basis for hormonal treatment (7-17). Levels of oestrogen, the administration of which is known to cause symptoms of painful nodularity, does not seem to be abnormal in CPM patients. Progesterone deficiency due to inadequate corpus luteum function is unlikely to be present. Defect in the tissue response or an end-organ abnormality is so far partially studied and needs further elucidation (15-16).

A third of mastalgia patients solicited treatment. Hormonal manipulation was done by danazol, tamoxifen, bromocriptine, progesterone, oral contraceptive pill, LHRH analogue (17). Non hormonal agents include analgesics, plant extracts like fructus-agni-casti, evening primrose oil and GLA. Randomized controlled trial of centchroman (ormeloxifene-SERM) 30/mg/weekX2 for 3 months resulted in abrogation of nodularity (93%) and highly significant of amelioration of pain. A highly effective with least side effects agent – ormeloxifene is now in regular use in several clinics in the country (18).

Of the prevalent treatments used for this condition; breast supporting garments, NSAIDs, Vitamins B6 and E, methyl-xantine (coffee) withdrawal, gamma linolic acid (GLA), progestogens have not found to be effective or better than placebo in randomized trials. Hormonal manipulations with dopamine agnostic agent that suppresses prolactin responses like bromocriptine are effective but have side effects and expensive. Similarly, treatment with danazol – a testosterone derivative causes hirsutism, muscular pain and loss of breast durity. Treatment with GnRH, tamoxifen and ormeloxifene (centchroman) are effective. GnRH is expensive and difficult to administer, tamoxifen is more commonly used in cancer therefore ormeloxifene now marketed in India for benign breast disorder with its least side effects is the treatment of choice.

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Ann Natl Acad Med Sci (India), **52(2):** 124-130, 2016

Innovations in Strengthening Medical Education in India

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ABSTRACT

There is a disconnect between the objectives of medical education in the country and the actual training being imparted. The present system of discipline based MBBS Curriculum has many inherent disadvantages eg. Compartmentalized teaching, poor development of problem solving skills, failure to generate interest in students and acquisition of dissociated knowledge are few of them. The **SPICES model** of medical education ie. (Student centered, Problem based, Integrated, Community oriented, Elective enabling and Systematic exposure) may be better suited to our country. Assessment system and examination system need a very drastic change based on the needs of the Community and the stakeholders in the healthcare section. Internship programme needs to be totally revamped. The acquisition of practical skills using newer medical education technology like **DOPS (Directly Observed Practical Skills)**, one minute preceptor and other newer methods needs to be incorporated. In our study on "DOPS" interns we found the usefulness of this methodology **(FAIMER study - Chhina RS)**.The use of technology has revolutionized the world eg. in Space technology, Computer Sciences, Social marketing Strategies. There is an urgent need to incorporate the "MOOC" model and the Social media eg. Facebook, Twitter, We chat, Whatsapp for better coverage and more useful teaching modules. In our study, we found "Facebook" teaching to be an important component of improving the teaching methodology and acquisition of knowledge by students **(FAIMER study- Sharma Anu & Chhina RS)**. The "Feedback" technique for improvement in the needs of student knowledge base, their aspirations, what they thought is appropriate in teaching skills and methodology was studied and powerful conclusions have been drawn in our

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DR. K.L. WIG ORATION delivered during NAMSCON 2015 at the All-India Institute of Medical Sciences, Patna.

institution. (**FAIMER study-Singh Daljit**). The postgraduate training seats needs to be modified as the disease burden load in the country requires. A study done by us showed a total disconnect between the need and the available resources in the State of Punjab. The requirements of the Community, Medical students, Healthcare providers and patients need to be advanced in an objectively scientific, need oriented manner in relation to medical education in India.

Key Words: Medical Education; DOPS (Directly Observed Procedural Skills); MOOC (Massive Open Online Course); FAIMER (Fellowship, Foundation for Advancement of International Medical Education & Research).

Innovation is a noun that means something new. Lot of debate is ongoing on the issue of the Medical Education in India. The State of Medical Education in India presents a scenario marked by rhetoric and wishful thinking rather than concrete steps in right direction. The search for a need based curriculum is not new. It has been felt for ages, but the curriculum has not really changed. It is an oft-repeated criticism that our medical colleges are producing graduates who are not well equipped to tackle the health care needs of the society (1).

The Medical Council of India (MCI) has recommended both horizontal (eg. Anatomy, Physiology, Biochemistry) and vertical integration (e.g. Anatomy with Surgery) to be introduced throughout the curriculum. The latest MCI guidelines stipulate that undergraduate medical education should be oriented towards health and community. Students training must aim at inculcating scientific temper, logical and scientific reasoning, clarity of expression, and ability to gather and analyze information (2).

The students in today's era of evidence based medicine favor “hard” clinical knowledge over “soft” health promotion/disease prevention, knowledge and skills. This has also been commented upon and discussed in the international forum like World Health Organization South East Asia Regional Organization (WHO SEARO) Health Minister's Meeting (3).

Learning Approach :

Students learn with the following three approaches commonly followed:

a. Superficial Learning
b. Deep Learning
c. Strategic Learning

Superficial learning is based on the approach of 'Recall' and the students without understanding of the facts memorize and this forms the base of Miller's Triangle knows and is the lowest level of learning.

Deep Learning is based on understanding of the facts and figures and also needs a comprehensive and critical

review and understanding of the subject. Deep Learning is needed for life long understanding and learning process that forms the basis of clinical practice in future. The “Knows How” of the Miller's triangle is based on this approach (Fig.1) (4).

Students are likely to adopt a deeper approach to their learning and achieve quality learning outcomes. When teachers provide for:

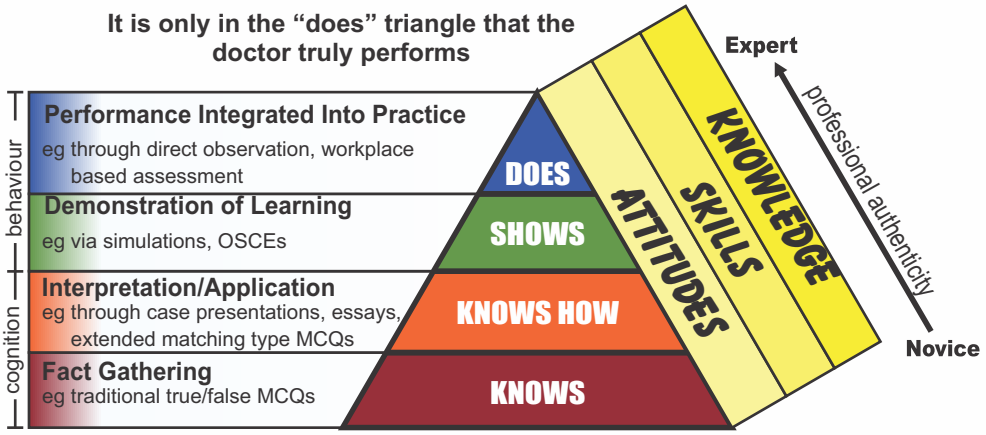
- Motivation and curiosity
- Student independence
- Student choice
- Opportunities to work with other people
- Low threat environment
- Challenging environment
- Frequent, constructive and supportive feedback
- An emphasis on higher levels of objectives.

The strategic learning approach inculcates a plan with the problem based learning. What is needed how much is needed and how it will help the top of the Miller's triangle 'Does' becomes an integral part of the learning experience.

The learning/teaching triangle shown below shows the learning percentage for different models of teaching (Learning Pyramid) (Fig.2).

From above it is clear that typical classroom teaching is inadequate for proper retention rates and modalities of teaching fare better (5).

Current medical education should prepare healthcare professional to be able to deal with the intricacies of healthcare system in addition to their clinical skills, economic knowledge and national healthcare needs have to be incorporated



Based on work by Miller GE, The Assessment of Clinical Skills/Competence/Performance; Acad. Med. 1990; 65(9); 63-67
Adapted by Drs. R. Mehay & R. Burns, UK (Jan 2009)

Fig. 1 : Miller’s Prism of Clinical Competence (aka Miller’s Pyramid)

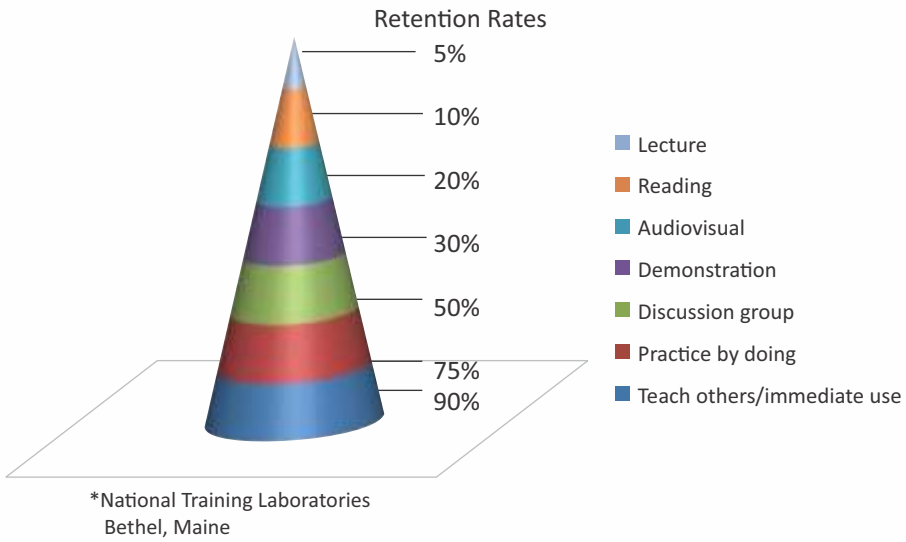


Fig. 2 : The Learning Pyramid*

into the medical curriculum. This change is needed at all levels and the sooner it is done, the better it is for the dynamics of medical education.

Edu-tainment :

Education and entertainment have to be combined and the rhetoric of medical education being boring, a repetitive and non-competency based is being debated for a long time. Medical Education has a history of tinkering with the medical curriculum without realizing larger educational objectives. Medical colleges have to create a true learner centered environment that makes active, self directed learning under the facilitation of interested faculty members a possibility. When Education and Entertainment are combined the word "Edu-tainment" emerges and true learning/life long learning occurs in the non-threatening, conducive, flexible and relaxed environment.

Innovations in Medical Education :

Innovations tried and validated by us have been in the following areas:

1. Teaching
2. Curriculum
3. Assessment
4. Use of technology

Teaching methodology is variable from regions, countries and person. Student centered teaching is the buzz words and following points are worthy of mention:

- Students are active in planning, rehearsing and assessing outcomes.
- Students make the choices on what and how to learn.

- Learning across the curriculum, flexible and can occur anywhere.
- Teacher is more of a mentor, guide and a facilitator.
- Emphasis is on a long time/ life long learning.

The acronym SPICES refers to six main concepts in Medical Education- student centered teaching, problem based learning, an integrated curriculum, and community based teaching, electives with a core and the use of systematic method. An awareness of these principles means the medical students can take a more active role in their learning. Students who are well informed about medical education principles, such as the SPICES criteria, are more likely to be able to provide constructive feedback about their own medical education experience, contributing in the long term to course (6).

Technology and Medical Eduaction :

Use of technology has revolutionized the medical education in it's various formats. Journal of Graduate Medical Education, June 2014 has article on the use of smartphones in "Graduate Medical Education" (GME). There are many ways that mobile phones applications (apps) can be used for medical education and bedside case to complement traditional in class teaching methods. Although the initial impulse of some educators might be that the smartphones, ipads and computer might be a distraction, but the myriad uses prompt us to consider the paradigm shift.

In "Smartphones, Trainees and Mobile Education: Implications for Graduate Medical Education," Short *et al* review the many ways that mobile phone applications (apps) can be used for education and bedside care to compliment traditional in classroom teaching approaches. Are we talking full advantage of teaching through mobile platforms? We propose that the "teachable moment" has expanded. iPads preloaded with digital resources, including textbooks and podcasts of lectures; the ipads also have the ability to interface with digital stethoscopes, portable ultrasound technology and encrypted electronic health records.

The following questions may be considered by the GME community:

- What are the most effective models of new technology?
- Can targeted learning be individualized using new technology?
- Are there metrics to determine the quality and validity of open access, online content in medical education?
- Can technologies be harnessed to assess competencies and milestones?
- How do we use technology to facilitate patient centered care (eg. Communication, patient education, shared decision making)?
- How can the electronic health record serve as learning tool for residents?
- What are the standards of professionalism and how does one teach them to learners in this new age of open, collaborative social media?

- Can we establish standards for sharing patient information via technology and social media?
- From the perspective of a “digital educator", how can we demonstrate scholarship for faculty.
- The concept of 'MOOC' needs to be emphasized (7).

Inspiring innovations in medical education what is the bright idea? Using facebook as a medium of interface in students has yield good results in our centre (Sharma Anu *et al*.).

Feedback and Medical Education :

- Feedback of students (Anurag Chaudhary *et al*.)
- Appraisal of undergraduate feedback (Hemlata Badyal *et al*.)
- Feedback of Teachers (Dr. Daljit Singh *et al*.)

We have conducted studies on the above three parameters and have reached a conclusion that a positive constructive feedback with scope of improving the outcomes remains the backbone for any system to progress. Anything which is assessed on a periodical basis is likely to show improvement over a period of time. The student/Faculty feedback improved our understanding of the complexity of student faculty interaction. When student feedback was appraised it showed a linear correlation with improved outcomes.

The Internship Year :

We have tried for the competency based medical education in interns. Internship is a phase of training when new graduate is expected to aquire skills under supervision, so that he/she may become capable of functioning independently. Often new graduates go through this period without a clear aim. We conducted an orientation programme before fresh graduates started their 1-year internship to familiarize them with their clinical tasks and their role in the community (8).

The internship is a very important part of educational training of Medical Student but it has deteriorated over time and it has reduced it's relevance and value to the students. The internship currently “UNASSESSED” does not serve the purpose of giving hands on experience because the intern's focus and target is the postgraduate examinations rather than acquiring the clinical skills.

Changes and innovation which have been tried:

a. Period must be assessed.
b. Time should be spent on acquiring and enhancing skills.
c. Skill development must ensure competence in delivering life saving measures.

The use of log books/ procedure books has been tried in our institution.

Structuring of training programme of interns in the department of medicine and assessment of procedural skills using

"Directly observed procedural skills" (DOPS) was used and the following conclusion were made that the use structured protocol for skill assessment by DOPS is helpful methodology for improvement of knowledge and procedural skills of interns.

CME in Undergraduates :

CME in Medical Education has been shown by us to be of value in the pre and post CME assessment of the knowledge and understanding of the subject by the students.

Thesis Writing :

Thesis writing as a protocol is being followed in the Country. It needs to optimized and in line with the Health Care needs of the society.

The downhill course in some aspects needs to be reversed. The prospects of medical education have a positive outcome provided, we do the needed changes at the earliest.

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Ann Natl Acad Med Sci (India), **52(2):** 131-138, 2016

High cervical myelopathy due to bony craniovertebral junction anomalies (atlantoaxial dislocation) in pediatric population- clinical scoring system

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SUMMARY

Bony craniovertebral junction anomalies are rare anomalies to cause high cervical myelopathy. Atlantoaxial dislocation (congenital) is one of the commonest bony anomaly in children presenting with high cervical compression. It is relatively common in India with an incidence of 5-8 / 1000. When the distance of atlas (anterior arch) is more than 3mm (4 mm children) from odontoid process, it is called as Atlantoaxial dislocation (AAD) resulting into bony compression of high cervical cord. The patients may present with quadriparesis, sensory impairment in all limbs along with lower cranial nerve involvement. Because of lower medullary involvement the respiratory compromises are also frequent, posing a threat to life. Complex anatomy of foramen magnum, plethora of clinical conditions and atypical surgical approaches are responsible for poor outcome in these children. A new clinical scoring system for myelopathy was evolved in order to have an objective and precise grading of these cases preoperatively and postoperatively. The need of precise scoring system was felt to have reproducibility and easy applicability in children of craniovertebral junction anomalies in order to fetch even minimal improvement or deterioration following complex surgery. Motor functions, gait, sensory, sphincteric, respiratory function & spasticity were the parameters included in study of scoring system. This study was done in 177 operated cases of AAD (67 patients, below 14 years of age included for statistical analysis). Their detailed clinical & radiological evaluation was done preoperatively & postoperatively. The Kumar & Kalra high cervical myelopathy grading system was thus, introduced in literature. System was easy to use, interpret and was more sensitive

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DR. S. JANAKI MEMORIAL ORATION delivered during NAMSCON 2015 at the All-India Institute of Medical Sciences, Patna.

to the changes in neurological status. It helped neurosurgeons and neurologists globally to evaluate and prognosticate the cases of Atlantoaxial dislocation.

Keywords : Pediatric, atlantoaxial dislocation, CV Junction anomalies, high cervical myelopathy.

The craniovertebral junction (CVJ) is a complex transition zone between the cranial cavity and the rostral spinal column. Various conditions affect and destabilize this region; of these conditions, congenital atlantoaxial dislocation (AAD) is the most common anomaly (1, 2). The management of congenital CVJ anomalies is complex due to the relative difficulty in accessing the region, the critical relationship of neurovascular structures, and the intricate biomechanical issues involved, which revolve primarily around the attainment of neural decompression and stabilization (3). The difficulty accessing the CVJ relates primarily to ventrally compressing pathologies in which the transoral procedure is required to take out the offending bony element. In comparison, posterior access to the CVJ does not pose much of a problem but has threat of neural injury.

The most common age group affected by congenital AAD is the second and third decade; however, the incidence in the paediatric population is not uncommon. A wide variety of congenital anomalies of the CVJ occur due to an insult of bony and soft tissue elements, between the fourth and seventh week of intrauterine life (4). The clinical manifestations are most often delayed into the second and third decade because they are usually subtle and easily missed in children unless looked for specifically (1, 5). Moreover, this disease process is slowly progressive and manifests due to chronic compression of the cervicomedullary junction; it is usually brought about by triggering events such as trauma (1, 5, 6). The criteria for diagnosis of AAD differ between children and adults. The normal atlas to dens interval is greater in children compared with adults; thus, AAD is diagnosed on the basis of atlantodental distance greater than 4 mm in patients younger than 9 years of age and greater than 3 mm for those 9 years of age or older (6). The management of these patients becomes even more difficult as a result of the general issues of managing children, which add to the typical issues of managing the complicated anomaly itself. There has been a progressive change in the approaches and techniques used for management (7). The outcome analysis performed to assess the surgical result has relied on Di Lorenzo's grading (DLG) system, which is a four-grade system based on functional dependence (Table 1) (3, 5, 8). This has been the most widely used and accepted grading system to

assess AAD patients undergoing treatment. The grading system has stood the test of time and has excellent reproducibility and inter observer reliability (7). However, although the DLG grading system is universally acceptable in adults, it may not be as readily applicable and useful in children. Children are unable to quantify their functional status and are otherwise dependent for their daily activities. Hence, it is difficult to access their functional disabilities caused by the disease process. Categorization according to disabilities as is done in the DLG may not be precise in this age group and lacks interobserver reliability and reproducibility.

Moreover, grading the patients into just four groups does not allow for the observation of subtle changes in neurological status. There is a large subset of patients who remain in the same grade even though they have subjective improvement or deterioration in their neurological status. It may be opined that a comprehensive and precise neurological examination may be more valuable in accessing the response to treatment. If a scoring system is developed that relies on these factors, it may be better reflective of progression of the disease course. Such a score should be detailed enough to recognize subtle neurological status changes but simple enough to be performed routinely. The purpose of the present study was to formulate a new scoring system based on comprehensive neurological examination and to assess its applicability in children compared with the DLG.

Table 1 : Di Lorenzo's Grading system

Grade 1	Independent, without deficits except hyperreflexia or neck pain. (Neurologically intact)
Grade 2	Independent for daily activities but having minor deficits (minor disability)
Grade 3	Partly dependent on others for their daily needs (moderate disability)
Grade 4	Totally dependent on others for daily needs (severe disability)

A study was done at SGPGIMS, Lucknow to introduce new scoring system; this was based on clinico-radiological evaluation of 177 cases operated over period of 14 years by single surgeon (RK). Statistical analysis was performed on children with age less than 14 years (n=67). The diagnosis was based on a minimum atlantodental interval of 4 mm. The patients who had associated Chiari- I malformation, AAD and associated genetic syndromes , or follow up of Less than 12 months were excluded. The assessment of patients was done preoperatively and postoperatively & follow up. The following points affecting the outcome were taken into consideration to fetch a new and precise scoring system

for better evaluation of children suffering from congenital AAD (Table 2). There were 36 males and 31 females, with a mean age 9.36±3.51 years (range 2-14 years). The results were compared with Di Lorenzo grading system.

Table 2 : Kumar & Kalra scoring system (K & K)

Parameter	**1**	**2**	**3**	**4**	**5**	**Total score**
Motor power	Contraction w/o movement or plegia	Movement with gravity	Movement against gravity	Movement against resistance	Normal power	5
Gait	Wheel chair bound or bed ridden	Restricted mobility despite aid	Mobility using aid	Slight disturbance, no aid required	Normal	5
Sensory involvement	Total loss of function	Restriction of function of daily living	Significant involvement (>25%) but no dysfunction of daily living	insignificant	No sensory loss	5
Sphincter involvement	Retention requiring indwelling catheter	Occasional CIC required with hesitancy	Hesitancy with residual urine not requiring catheter	Hesitancy but no residual urine	Normal	5
Spasticity	Affected part rigid in flexion or extension	Passive movement difficult	Passive movement easy	Slight increase in tone	Normal	5
Respiratory difficulty	Requires assisted respiration	Dyspnoea at rest	Dyspnoea on mild exertion	Dyspnoea on moderate exertion, unable to do active work	Normal	5

Disability Grading :

67 patients, treated surgically for AAD and having age less than 14 years, included in this study. They were assessed with the DLG and Kumar and Kalra (K&K) scores pre-and postoperatively (Table 3 and 4). The patients were divided into four grades according to the K&K scores, with grade 1 patients having score from 25 to 30, Grade 2 patients having score from 19 to 24, Grade 3 patients having score from 13 to 18 and Grade 4 patients having score from 6 to 12 points. Most of the patients had poor grades. Using the DLG scoring system, there were 26 (38.8%) patients in Grade 4 and seven (10.4%) patients in Grade 3 preoperatively. The maximum postoperative improvement was witnessed in the Grade 4 patients, with as many as 23 patients showing improvement from their preoperative grades. Overall, 47.8% of the patients showed improvement and 10.4% showed deterioration. There was a high percentage (41.8%) of patients who showed stabilization of their grades. When the K&K scoring system was used on these patients, most (65.7%) of the patients were rated as Grade 2 preoperatively. The number of patients (n=13) in preoperative Grade 1 was also high. Only three patients were rated as Grade 4 preoperatively, and none remained in this group postoperatively. Overall, improvement was observed in the majority (n = 55) of the patients; fewer patients had deterioration (n = 6). Thus, stabilization of neurological status was seen in 41.8% of patients using the DLG score and in only 8.9% of the patients using the K&K score. Although 47.8% of the patients showed improvement using the DLG scoring system, a very high percentage (82.1%) of patients showed improvement using the K&K score. Out of the 28 patients who had shown stabilization of their neurological status using the DLG score, 23 patients showed an improvement in scores when assessed by the K&K score, two patients showed the stabilization of their scores, and three had deteriorated from their preoperative scores. The minimum improvement was observed in those with symptoms of weakness (n = 49) and spasticity (n = 47), which were the earliest symptoms to improve. The symptoms most resistant to treatment were respiratory (n = 7) and sphincter symptoms (n = 6). To establish the objective criteria of reliability and reproducibility of the proposed score, we analyzed each patient's DLG and K&K scores preoperatively and compared them with their most recent follow-up scores and outcome. The statistical significance was ascertained by calculating p-value using logistic regression and by measuring the positive predictive values for each score. The results are listed in Table 5.

Table 3 : Outcome analysis based on Di Lorenzo's grading system

Grade	Pretreatment, n= 67 (100%)	Posttreatment n=67 (100%)	Improved (%)	Same (%)	Deteriorated (%)
I	10 (14.9)	17 (25.4)	0	8	2
II	24 (35.8)	19 (28.4)	8	14	2
III	7 (10.4)	25 (37.3)	1	3	3
IV	26 (38.8)	6 (9)	23	3	0
Total			32 (47.8)	28 (41.8)	7 (10.4)

Table 4 : Outcome analysis based on Kumar & Kalra score

Grade*	Pretreatment, n= 67 (100%)	Posttreatment n=67 (100%)	Improved (%)	Same (%)	Deteriorated (%)
I (25-30)	13 (19.4)	17 (25.4)	11	1	1
II (19-24)	44 (65.7)	45 (67.2)	34	5	5
III (13-18)	7 (10.4)	5 (7.5)	7	0	0
IV (6-12)	3 (4.5)	0	3	0	0
Total			55 (82.1)	6 (8.9)	6 (8.9)

*Numbers in parentheses in the first column represent the value of the total score using the Kumar and Kalra score.

Table 5: Predictability of score

Score used	Mean pretreatment	Mean follow up value	P value	Exp (B)	Predictive value (%)
DLG	2.73 $\pm$ 1.14	2.30$\pm$ 0.95	0.714	1.094	52.2
K&K	19.91$\pm$ 4	23.22$\pm$ 3.36	0.000	0.218	82.1

DLG- Di Lorenzo's grade; K&K- Kumar & Kalra score.

The incidence of AAD in pediatric age group is significant enough to deserve special and separate emphasis. Majority of children with AAD in our country reach neurosurgeons through referral system and thus usually present in poor grades. After surgery, it has been observed that although majority of them show subjective improvement, a large number stay in same grade as their pre operative grade when DLG scoring system is used for assessment (1, 5). Furthermore there are patients who deteriorate & may require surgery but stay in same grade. The DLG score used for adults is lacking in its reproducibility in children and does not truly reflect the gravity of disease process. Thus, it is not a very sensitive indicator of change in neurological status and cannot be reliable in children. The change in score and neurological status is better reflected by K&K scores rather than

DLG. Positive predictive value was better using K &K score , and outcome analysis using K&K score was statistically significant whereas the changes didn't bear statistically significant correlation using the DLG score (p=0.714). The advantages are the assessment and comparison of neurological status based on commonly occurring symptoms and signs, which are routinely assessed in neurological examination of such patients. The individual symptoms and signs can also be analysed and compared individually in terms of their severity and significance. Thus, both an overall status of function and individual symptom is known. The score is based on multitude of factors and hence is more sensitive. It is also more suited for statistical analysis. The score may very well be used for adult patients as the parameters used are common to adults also. Furthermore, after exclusion of respiratory parameters, it can also be used for myelopathy in other areas of spine.

To conclude, K & K scoring system is easy to use and interpret, and is more sensitive to the changes in neurological status of patients than the currently used DLG system (9).

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