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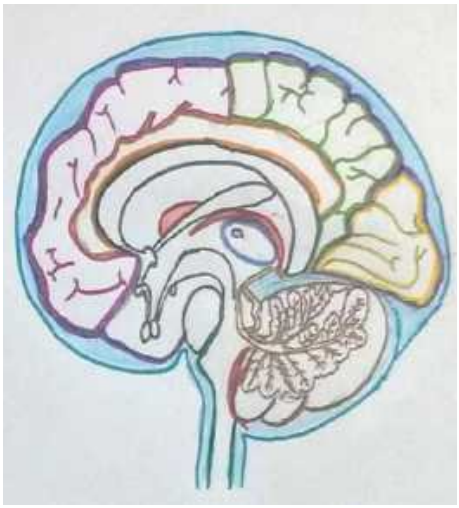

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CONTENT

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From the Editor's Desk

Dear Readers,

This issue brings out two Original Articles from the department of SLHS and Faculty of Pharmacy, two Review Articles and two Case Reports.

Editorial article highlights the importance of sample size in Medical Research.

The Journal provides a platform to motivate young researchers to publish and spread the knowledge about their work to wider spectrum of scientists. Please use the forum to publish more articles in our journal.

P.V. VIJAYARAGHAVAN
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Original Article

"ISPEAK"- AUGMENTATIVE AND ALTERNATIVE COMMUNICATION FOR CHILDREN WITH COMMUNICATION DISORDERS.

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ABSTRACT

Background and Objectives : *Augmentative and Alternative Communication (AAC) includes all forms of communication (other than speech) that are used to express thoughts, needs and ideas. People with severe speech or language difficulties rely on AAC to supplement existing speech. This increases social interaction, school performance, and feeling of self esteem. Studies in the past have mentioned greater results in the usage of AAC. This study focused on the development of a prototype AAC "ISpeak" which is cost efficient and can be easily maintained.*

Method : *This device was administered on forty children with various communication disorders and whose language age ranges from two to four years. The materials like controller board, voice board, wires, push buttons, mica sheets, coloured naturalistic pictures and speaker were used to device ISpeak. It was used to assess the accessibility and easy usage of the device.*

Results : *Five domains such as water proof, durability, proximity, power supply, stimuli were tested and positive results on all five domains were obtained.*

Conclusion: *This high tech AAC I Speak will be useful in assessment and intervention of children with non-verbal communitcations.*

Key words *: Augmentative and Alternative Communication, Communication disorders, ISpeak.*

SRJM 2016;9: 1-4

INTRODUCTION

Alternative and Augmentative Communication (AAC) is defined as an area of clinical practice that attempts to compensate (either temporarily or permanently) for the impairment and disability patterns of individuals with severe expressive communication disorders".[1] AAC system can be broadly divided into two types: aided and unaided communication systems. The unaided communication systems are those which rely on the user's body to convey messages like gestures, body language, and/or sign language. The aided communication systems are classified as low tech AAC devices, which range from papers to picture cards. They are often less intimidating for the listener and are lighter in weight to be carried anywhere. The lack of speech output proves to be a big restriction for some users of low tech device. High tech AAC devices, ranges from communication boards to devices that produce voice output, programmed to produce different spoken languages. There are high chances of breakdown or damage of these devices. A variety of AAC devices are available in the market, that is both high and low tech. One such device is "Avaz" which is a high tech AAC, available in India. An individual has complex communication need and are limited in using their vocabulary in a range of settings, it is essential to utilize a well thought-out system, which is tailor-made for the individual's needs and environments.[1] Currently limited materials are available which could be tailor made for individual child's need.

Children with Cerebral Palsy, Mental Subnormality, Down's Syndrome, Autism Spectrum Disorder, Childhood Apraxia and other childhood communication disorders are beneficial with the use of an AAC device.[2] 68% children with communication disorders are benefitted from the use of AAC in United States. It was also observed that over 11% of children enrolled in special education require AAC.[1] Children with Autism Spectrum Disorder have shown impact on communication using AAC applications. Also, it is important to carefully design the AAC depending on various communication needs. The accessibility of these applications by social and economically backward regions is limited.[3] Though the current scenario of our country is marching towards technological developments in this field, language and social behaviour of children with communication disorders will be enhanced through the usage and training of AAC.[4] Thus, there is a need to develop an AAC system which is cost efficient and easily procurable by all sections of the society. The aim of

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this study is to develop a high tech AAC device which is cost efficient and has low maintenance.

MATERIALS AND METHODS

Material development

The material was developed based on reviewing various literature search and different models of AAC available in the current market/scenario in India. There are different categories of AAC, from low tech to high tech devices. This study focussed on development of a prototype AAC which was tailor-made for an individual child's communication needs. The ISpeak was constructed using electrical materials such as wires, integrated circuits, a voice board and a controller board. The device was developed using coloured pictures of basic lexical categories and verbs in the form of stickers of 2B size. These materials were procured and assembled with the help of an electronic professional. A total of six pictures were uniformly arranged on the AAC board. The board was fixed with eight push buttons for each stimuli present. Each button corresponded to the respective stimulus linked to the voice output. There were a total of sixty two speech stimuli which was recorded using a female voice. The board has a microphone that can record any new vocal stimulus for further modification in vocal output. Also, a speaker was mounted on the device for vocal output. The power supply for the device was provided through an adapter. The sharp edges of mica sheets were covered with colourful tapes to avoid injuries while using the AAC board (Fig.1).

A pilot study was done on five children between the age range of 6 - 7 years with the language age of 2 - 3 years (Mean age: 2years, 5months) from an early intervention centre. Prior permission from the school head was obtained. Parents were explained about the study and informed consents were obtained. All these children had language delay with no problems in locomotion. All children had Tamil as their native language (Table 1).

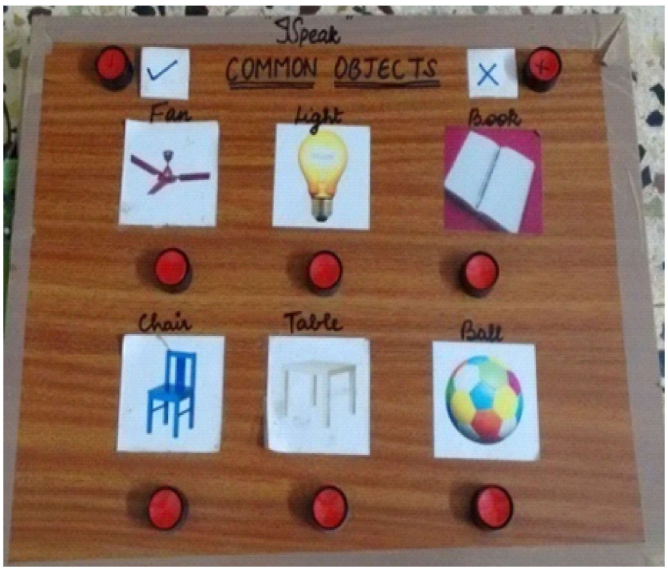

Fig.1: Assembled *ISpeak*

Table 1: Demographic and clinical details of the participants

Sl. No.	Participants	Chronological age/ sex	Language age	Provisional diagnosis
1	Child 1	6/M	2;2	Intellectual Disability
2	Child 2	6;3/F	2;6	Autism Spectrum Disorder
3	Child 3	6; 7/M	2;4	Pervasive Developmental Disorder
4	Child 4	6;5/M	2;2	Attention Deficit Hyperactive Disorder
5	Child 5	6;9/F	2;9	Intellectual Disability

Each child was allotted three sessions of training. On the first session language evaluation for each child was done using Assessment of Language Development (ALD).[5] AAC protocol was administered on each child for the following skills: sensory skills, communication skills, physical abilities and positioning. Based on this evaluation, the pictures were selected and were tailor made for every child. In the second and third sessions, the children were trained individually to operate the device. Since these children were already using a low tech AAC, it was easy for them to adapt with the procedure. They were trained to press the appropriate push button when asked for the target object. The

Table 2: Profile of Clinical disorder in participants with non-verbal communication

Sl. No.	Disorders	No. of participants
1	MRELD* with Autism Spectrum Disorder	10
2	MRELD with Intellectual Disability	10
3	MRELD with Down's Syndrome	10
4	MRELD with Cerebral Palsy	5
***MRELD: Mixed Receptive and Expressive Language Disorder**		

following domains like easy usage, proximity of pressing the buttons, portability, weight of the device, short circuiting, waterproof, power consumption, were tested and the results were noted. Most of the domains showed positive results. Few modifications such as decreasing the size of the board, placing an inbuilt speaker were added in the device.

PROCEDURE

Participants

Thirty five children with language delay (10 children with Autism Spectrum Disorder, 10 children with Intellectual Disability, 10 children with Down's Syndrome, and five children with Cerebral Palsy) were included in the study. These children were selected between the age range of 6 and 7 years with the language age ranging from 2 to 4 years from an early intervention centre. Prior permission from the school in charge was obtained. Parents were explained about the study and informed consents were obtained. All children had Tamil as their native language (Table 2).

Assessment

The AAC administration was done in the same manner as that of pilot study. All the sessions were video recorded using Samsung SRD - 1670 DC. The parent and the clinician were present during the assessment. Each session lasted for 45 minutes. The assessment was carried out on comprehension of common objects. Children were trained to comprehend the stimuli and how to use the device for eliciting a response. After this training session, the stimulus was presented to the children and they pressed the respective buttons. Domains like water proof, durability, proximity, stimuli and power supply were tested and the results were noted.

Analysis

All the sessions were video recorded and were individually analysed. Descriptive statistical analysis was used and the results were noted for each domain for different communication disorders.

RESULTS

Domain specific observations

On observation for waterproofing, 88% of children had no issues. None of the children had any problem with short circuiting. Under the domain, durability of the material, 93% of the children had good response. None of them had any issues in the power supply to the device. In proximity of pressing the buttons, 85% of them showed good responses. All of them perceived the stimuli appropriately, since coloured naturalistic images were used.

Disorder specific observations

Three children with Intellectual Disability had drooling on the device, which was wiped off easily with the tissue. Four children with Autism Spectrum Disorder pushed the AAC board down the table three times and the device remained sturdy. Two children with Cerebral Palsy showed excellent proximity of pressing the buttons since the buttons had boundaries and the click sound when pressed gave an auditory feedback for accuracy.

Maintenance of the device

the device can be easily maintained by replacing the buttons when they are damaged by procuring the buttons available in the market. In case if the circuit in the device breaks, it can be fixed by an electrical or electronic person. The stimuli can be pasted on the device when they are detached. New vocal stimuli can be added at any point of the time by the care taker.

DISCUSSION

This study focused on developing a prototype AAC ISpeak. The results revealed that this device was easily adaptable by children of different communication disorders. Since coloured and naturalistic pictures were used, children were interested to participate in the session. Children were able to perceive coloured images quicker than black and white images when arranged before them.[5].

ISpeak produces speech output which provided a positive reinforcement for these children. It also has an added feature of recording a new voice, based on the child's need. It was observed that when there is a speech output in a device, there is a reduced demand for motoric activities. Hence, it results in reduced stress level on all systems and increased speech production.[6]

The device had buttons of same colour and the proximity of reaching the switches showed good results. The buttons also provided a tactile feedback for all children due to boundaries in the switches. The size of the switch shouldn't be too large or too small. The user should feel the touch of the switch, the pressing sound provides accuracy for user and colour of switch should be same throughout the device.[7]

Some of the issues related to ISpeak are that, it requires continuous power supply and the portability of the device which hindered the usage of device in

different environment. The children needed caregiver's assistance to switch on the device. These can be modified in future research. Thus, it can be used as an effective clinical tool for treating children with communication disorders.

CONCLUSION

The high tech AAC ISpeak can be used as an intervention tool for various communication disorders. It is useful in assessment and intervention of children with non verbal communication. A longitudinal study on a large population with different communication disorders will provide the efficacy of usage and success in the intervention.

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PERSPECTIVES OF PHARMACOVIGILANCE OF AYUSH DRUGS AMONG HEALTH CARE PROFESSIONALS - A CROSS-SECTIONAL SURVEY

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ABSTRACT

Objective: *To assess the knowledge of Pharmacovigilance of AYUSH drugs and recommendations for improving adverse drug reporting of AYUSH drugs among the healthcare professionals working in various disciplines.*

Methods: *This study was a cross- sectional questionnaire based survey conducted during a one day national seminar on "Pharmacovigilance of AYUSH drugs" conducted by Faculty of Pharmacy, Sri Ramachandra University. A structured questionnaire was designed and distributed among 500 delegates working in different districts of the state of Tamilnadu. The colleceted data were analysed using Epi Info software and expressed in percentage (%).*

Results: *Analysis of the data revealed that 36% were aware of the existence of the National Pharmacovigilance Program for AYUSH drugs in India, 24% knew that the National Pharmacovigilance Centre is located at Indian Pharmacopoeia Commission (IPC), Ghaziabad, 12% were aware that the International Centre of Pharmacovigilance is located at UPSALA, Sweden, 14% were familiar with a standardized form for Reporting ADRs of AYUSH drugs and 12% knew that most commonly used causality assessment scale is Naranjo's scale.*

Conclusion: *The present study revealed the lack of knowledge of pharmacovigilance of AYUSH drugs among the study population. There is a need for a regular training and the reinforcement for the ADR reporting among the health care personnel for the better clinical management of the patients in general.*

Keywords: *Pharmacovigilance. Adverse Drug Reaction, AYUSH.*

SRJM 2016;9:5-9

INTRODUCTION

Pharmacovigilance is defined as the science and activities relating to the detection, assessment and prevention of adverse events and all other problems related to medicines. Moreover, it has a vital role in therapeutic decision-making, either for an individual or national or in global perspective.[1]

More than 60 to 70% of modern medicines in the world market are directly or indirectly derived from plant products. The common myth regarding herbal medicines is that these medicines are completely safe and can therefore be safely consumed by the patient on his/her own, without a physician's prescription.[2] This belief has led to large-scale self-medication by people all over the world, often leading to disappointing end-results, side-effects, or unwanted aftereffects. The National Pharmacovigilance Program was launched in India keeping in view of the increasing global concern regarding safety of Ayurvedic drugs.[3,4]

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Several recent high profile herbal safety concerns, such as renal failure and urothelial cancer associated with exposure to Aristolochia species, allergic reactions, skin inflammation with garlic, allergic dermatitis with aloe vera and hepatotoxicity associated with kava-kava have contributed to the increasing awareness of the need to monitor the safety of herbal medicines.[5,6,7]

With this background, the present study had an objective to assess the awareness about Pharmacovigilance of AYUSH drugs among the health care professionals and to discuss various ways to make it operationally better among the health care professionals and promote a culture for reporting regularly to the respective peripheral or higher centers.

MATERIALS AND METHODS

This cross-sectional survey was conducted during the National seminar on "Pharmacovigilance of AYUSH drugs" organized by Faculty of Pharmacy, Sri Ramachandra University on 19th January 2016. A list of 604 delegates from various disciplines including research scholars and students from AYUSH and pharmacy colleges, research scholars and officials from the Central Research Institute for Siddha and Central Research for Ayurveda, teachers from pharmacy and other life sciences institutions and

Supplement-1

NATIONAL SEMINAR ON PHARMACOVIGILANCE OF AYUSH DRUGS		
QUESTIONNAIRE		
Name (Optional): Designation: Qualification:		
Are you aware of existence of National Pharmacovigilance Program for AYUSH drugs in India?	Yes	No
Where is the National Pharmacovigilance Center for AYUSH drugs situated?	IPC / Ghaziabad / Kolkata / New Delhi / Pune	
Where is the International Pharmacovigilance Center for AYUSH drugs situated?	USA / UK / Sweden / Australia	
Have you ever encountered any adverse event with AYUSH drugs?	Yes	No
Have you ever reported any ADR?	Yes	No
a. If YES How many		
b. If not, what is the reason?		
Are you familiar with standardized form for Reporting ADRs of AYUSH drugs?	Yes	No
Do you know the commonly used scaleto establish the causality of an ADR?	Yes	No
a. If Yes, Can you name it?		
Can you give an example(s) of any AYUSH drug(s) banned due to ADRs?		
a. AYUSH drug name		
b. Adverse event		
Have you attended any CME or training program about Pharmacovigilance of AYUSH drugs?	Yes	No
a. If Yes, Can you name it?		
What are your suggestions for improving ADR reporting of AYUSH drugs?		

health care practitioners of AYUSH drugs had participated in this seminar.

A structured questionnaire (Supplement-1) was distributed to the participants after the inaugural and before the session starts (pre-seminar). At the end of the session, the same questionnaire was circulated to all the participants (post-seminar). Data were expressed as N (%). Statistical difference between pre and post-seminar response was calculated using paired t-test in Graph Pad Prism.

RESULTS AND DISCUSSION

A total of 500 questionnaires were distributed to the participants after the inaugural and before the session starts, out of which 358 were returned to us for evaluation. Out of 358, we could able to evaluate only 326 as 32 forms were rejected for various reasons such as incomplete information, overwriting, tearing, etc. Thus the response rate before the seminar (Pre-seminar) is 65.20%.

With respect to knowledge part of the

questionnaire, 117 (36%) participants were aware of the existence of the National Pharmacovigilance Program for AYUSH drugs in India, 78 (24%) knew that the National Pharmacovigilance Centre is located at the Indian Pharmacopoeia Commission (IPC), Ghaziabad but an equal number of respondents said that the National Pharmacovigilance Centre is located at AIIMS, New Delhi. Only 39 (12%) respondents knew that the International Centre of Pharmacovigilance is located at UPSALA, Sweden, rest reported other countries.

Merely 46 (14%) participants were familiar with a standardized form for reporting ADRs of AYUSH drugs and thirty nine (12%) participants knew that most commonly used causality assessment scale is Naranjo's scale.

At the end of the session, the same questionnaire was circulated to all the participants and we received 142 forms for evaluation and the response rate at the end of the seminar (post-seminar) is 71.42%. When compared with pre-seminar, statistically significant improvement in response was observed for all the questionnaires in post-seminar evaluation (Table-1).

On accessing the familiarity and recommendations for improving ADR reporting by participants, only 12 (8.45%) respondents encountered adverse event with AYUSH drugs and merely 3 (2.12%) participants reported ADR. Maximum respondents i.e. 53 (37.32%) suggested that more advertisement is required about the pharmacovigilance of AYUSH drugs for improving ADR reporting of AYUSH drugs, 36 (25.35%) told more seminar or workshop on pharmacovigilance of AYUSH drugs need to be conducted, 24 (16.9%) said that pharmacovigilance should be a part of the

Table-1: Knowledge of Pharmacovigilance of AYUSH drugs by participants

Questions	Pre-seminar		Post-seminar		p-value
	n (326)	%	n (142)	%	
Are you aware of existence of National Pharmacovigilance Program for AYUSH drugs in India?					
Yes	117	36	142	100	<0.001
No	209	64	-	-	<0.001
Where is the National Pharmacovigilance Center for AYUSH drugs situated?					
IPC, Ghaziabad	78	24	80	56	<0.001
Kolkata	137	42	35	25	<0.001
New Delhi	78	24	27	19	<0.001
Pune	33	10	-	-	<0.001
Where is the International Pharmacovigilance Center for AYUSH drugs situated?					
USA	117	36	17	12	<0.001
UK	111	34	11	8	<0.001
Sweden	39	12	114	80	<0.001
Australia	59	18	-	-	<0.001
Are you familiar with standardized form for Reporting ADRs of AYUSH drugs?					
Yes	46	14	105	74	<0.001
No	280	86	37	26	<0.001
Do you know the commonly used scale to establish the causality of an ADR?					
Yes	39	12	37	26	<0.05
No	287	88	105	74	<0.01

curriculum and 18 (12.68%) participants did not respond (Table-2).

There is an ongoing problem with unexpected toxicity of herbal products due to quality issues, including use of poor quality herbal material, incorrect or misidentified herbs, incorrect processing methods, supply of adulterated or contaminated herbs or products.[8,9] These quality issues can be addressed to some degree by improved regulation requiring GMP standards for manufacturing. However, medicinal herbs/products come from many countries with differing manufacturing standards and enforcement of regulations. Hence, poor quality products are likely to remain a problem.

By recognizing the growing importance of the use of herbal medicines worldwide, World Health Organization (WHO) and AYUSH developed guidelines for the monitoring of herbal safety within the existing pharmacovigilance framework.[10] The First National Consultative meets of the National Pharmacovigilance Program for ASU Drugs was organized by the department of AYUSH, Ministry of Health and Family Welfare, New Delhi on August 2008, sponsored by WHO, where the draft protocol was technically reviewed and finalized. Based on the feedback received, final version of the protocol is prepared and the same is being released as a part of launching of the National Pharmacovigilance Program.[11,12]

Table-2: ADR familiarity and recommendations for improving ADR reporting by participants

Question	Response (n)	%
Have you ever encountered any adverse event with AYUSH drugs?	Yes (12)	8.45
	No (130)	91.55
Have you ever reported any ADR?	Yes (3)	2.12
	No (139)	97.88
What are your suggestions for improving ADR reporting of AYUSH drugs?		
a. Pharmacovigilance should be a part of the curriculum	24	16.9
b. More seminar/workshop should be conducted	36	25.35
c. More advertisement is required	53	37.32
d. All the hospital should have Pharmacovigilance department	11	7.75
e. No response	18	12.68

The purpose of pharmacovigilance programme is to identify the ADRs in large populations, establish new and rare ADRs, record the frequency and to implement measures for further prevention of these ADRs.[20] Numerous studies on Knowledge, Attitude and Practice (KAP) of pharmacovigilance have been conducted in various parts of India, however, no such study was conducted in our region, thus we decided to undertake this observational survey. The response rate in our study was 65.2%. It was almost similar to other studies by Desai et al,[13] Hardeep et al,[14] and Agarwal R et al,[15] all of which showed a response rate of 61%.

Only 36% participants were aware of the existence of the National Pharmacovigilance Program for AYUSH drugs in India and 24% knew that the National Pharmacovigilance Centre is located at IPC, Ghaziabad. This knowledge was scarce in our health care professionals as compared to other studies of Ramesh M et al,[16] and Ghosh S et al,.[17]

In our study, a small number of respondents, 46 (14%) participants were familiar with a standardized form for reporting ADRs of AYUSH drugs and thirty nine (12%) participants knew that most commonly used causality assessment scale is Naranjo's scale. These figures show poor knowledge of our staff as compared to the study of Agarwal R et al. [15] All these parameters clearly show deficient knowledge about pharmacovigilance and ADR reporting among doctors, residents and nurses in our college for which we need to employ suitable measures to improve the knowledge and awareness at every level.

A total of only 8.45% respondents encountered adverse event with AYUSH drugs and merely 2.12%

participants reported ADR in our study. This data were almost similar to the studies of Desai et al,[13] Pimpalkhute SA et al, [18] Sharma S et al [19] and Ghosh S et al. [17]

CONCLUSION

Our study revealed the overall lack of knowledge about pharmacovigilance and ADR reporting of AYUSH drugs among the health care professionals. However, most of the healthcare professionals showed a favourable attitude towards ADR reporting and were also enthusiastic to learn and practice it. Thus, the study recommends for more sensitizing programs, advertisement about ADR reporting at grass root health care system. This step will not only promote ADR reporting, but also will be helpful in reducing overall economic burden of health care cost, morbidity & mortality.

CONFLICT OF INTEREST

The authors have none to disclose.

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REVIEW - A FORWARD-THINKING: NEW METHODOLOGIES TO UNDERSTAND DISEASE BIOLOGY IN HUMANS

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ABSTRACT

It is the goal of all medical researchers to use as few animals as possible. Ultimately it would be ideal if the use of animals could be totally replaced by non-clinical methods. In the last 20 years, the number of animals used annually has decreased significantly and the search for validated alternatives continues. It is a common misconception that animals are used because they offer a "cheap alternative" to non-animal techniques. The reverse is in fact true. Research animals are very expensive to purchase, house, feed, and care for. Computers and laboratory equipment in the long run would be much less expensive, and much easier to care for. Many areas of research have already committed to replace all animal use with scientifically better alternative methods. Examples include the use of modern in vitro methods to replace the testing of caustic chemicals and the production of monoclonal antibodies, both procedures that are known to cause great pain when done on live animals. The main aim of this article is to focus on non-animal models, even though it cannot completely eliminate the use of animals in testing. However, several non-animal models have helped reduce the number of animals used in the field of biomedical and pharmaceutical research.

Key words: Animal, Alternative, Drug testing

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INTRODUCTION

Alternatives to animal testing was a substitute method for animal testing, which are used to develop new methods and performance of non-animal testing methods avoids the use of live animals.[1]

"Alternatives" or substitute is defined as replacement of laboratory testing animals in research, for the purpose of minimizing the level of stress endured by the laboratory testing animal.[2]

In 1995 Russell and Burch introduced the definition of alternatives as the 4 R's - Replacement, Reduction, Refinement, and Responsibility. These ideas at the moment are utilized in most of the upcoming research field.[3]

1. Replacement: Animal model to be replaced by non-animal techniques whenever possible.
2. Reduction: Reduction includes usage of less number of animals.
3. Refinement: refers to techniques that alleviate or de-crease capability ache, suffering or distress and enhance animal welfare for the animal's used.[1]
4. Responsibility: it is the additional responsibility to the unique 3 R's. It has grown into a new generation of overall performance, primarily based outcomes, which displays integrity, honesty, and clinical correctness in the proper and reasonable use of laboratory animals. This guarantees that animal existence is needed and necessary for biomedical development.[3,4]

In this review, the different disciplines of biomedical and pharmaceutical research are surveyed in order to focus the observation on disease biological studies on human by alternative testing methodology with minimum use of animal models.

What are those alternate testing methods or animal substitute?

Living testing methods can be

* Proteomics
* Genomics
* Tissue or organ culture
* Micro-organism

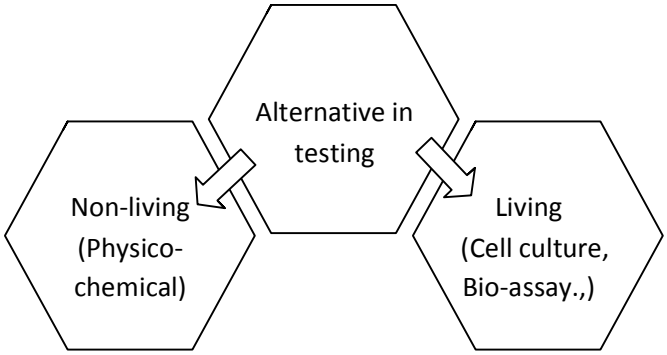

Fig 1: Classification of alternative animal testing models

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* Micro dosing
* Bio-assay.[1,2,4,6,7,8]

Non-living testing methods can be

* Physico-chemical techniques
* Computer stimulation or Mathematical data
* Non-invasive imaging techniques
* Molecular docking studies
* Micro-array
* Pyrogenicity

Living testing methods can be Research on '-omics' methods - which include genomics, transcripto-mics, proteomics and metabonomics focused on trying to identify a critical alternations in the genomic, proteomic or metabolic activities. This would lead to understanding how a chemical might damage genetic code or disturb the functionality of a cell.[33]

1. Proteomics

"Proteomics" is part of drug discovery or testing technology. Proteomics is the investigation of the proteome and includes the innovation used to distinguish and measure the different proteins, protein-protein and protein-nucleic corrosive collaborations inside of the proteome, and in addition to the post-translational alterations that influence protein movement. Proteomics innovations with computational strategies have been progressed as of late over numerous other corresponding methods. This empowers researchers to screen extensive quantities of proteins inside clinically unmistakable specimens that finds sickness bio-markers, recognize and approve drugs targets, outline more successful medications, appraisal of medication viability and patient reaction, i.e., to meddle with practically every progressions in cutting edge drug revelation process. Proteomic methodology of medication revelation incorporates finding a precarious protein that is creating an undesirable influence and afterward use of a particle to alter its impact. Proteomics joins parts of science, designing and data science and apply them to all regions of medication revelation. Presentation of more secure, more successful and savvier medications will be a definitive result of change of this innovation.[34,35]

2. Genomic

Pharmacogenomics is a study of how the drug is responding to a particular person gene. Objective of the pharmacogenomics is to optimize the drug therapy with respect to patient gene. This technique involves gene sequencing, gene analogy analysis, etc. Main focus of pharmacogenomics is single drug- single gene interaction. Pharmacogenomics is applied on several area of medicine such as oncology, forensic pathology, cardiology etc.[33]

3. Tissue or Organ culture

Tissue or Organ culture is a wide concept. On drug testing point of view tissue or organ culture gives the details about pharmacological and toxicological information on new drug such as the LD50 value of drug substances. It is the most successful method because in animal model, 50% of animal was killed and remaining animals are getting severe pain. Human cell was artificially grown or maintained in sterile culture media containing cell growth regulator and promoter of known concentration so that we get the details regarding absorption, distribution and bio-transformation of drug product. By using this technique, scientist can get the most accurate and faster result.[36,37]

4. Micro-organism

In pharmaceutical drug testing point of view, microorganisms are used to evaluate the efficacy, safety and potency of drug product. Broad spectrum anti-biotic of gentamycin sulphate drug potency was tested by using Staphylococcus epidermis, Bacillus pumilus. Even the vitamin cyanocobalamin also tested by micro-biological method. Antibiotic susceptibility test (AST) is to determine which antibiotic will give successful treatment for bacterial infection in vivo.[32]

5. Micro-dosing

This is a method to study the behavior of drugs in humans. It is done before Phase I clinical trial to find whether the drug is efficient for the next level of testing. Micro-dosing is used to decrease the resources spent in ineffective drugs and the frequency of testing which is done on animal models. In phase I clinical trials 40 percentage of drug will fail, which takes 18 months and it is expensive. By using micro-dosing, screening of drugs is done prior, results are obtained faster and are less expensive.

Micro-dosing requires 4-6 months, inexpensive and it shows excellent accuracy at predicting human metabolism. It is reported that largest pharmaceutical companies have used micro-dosing now-a-days in drug development. Micro-dosing has been provisionally endorsed by the USFDA and European Medicines Agency.[35]

6. Bio-assay

Bio-assay is a type of scientific experiment which is conducted to measure the effect or potency of drug

substance and it's compared with standard one by using of live animal part and it also used to find out the concentration of a particular constitution of a mixture.[36]

"The determination of the relative strength of a substance (e.g., a drug or hormone or toxicant) by comparing its effect on a test organism with that of a standard preparation" is called bioassay.[33,34]

When compared to other methods of assay such as chemical or physical, bio-assay is less accurate, less elaborate, more laborious, more troublesome and more expensive. The active principle of the drug is unknown or cannot be isolated or chemical method not available or the chemical composition is not known, or chemical composition of drugs differs, but have the same pharmacological action. Hence bio-assay is very useful method to evaluate the drug substance and determine the relative strength of drugs.[37,38]

NON-LIVING ANIMAL SUBSTITUENTS METHODS

1. Physico-chemical techniques:

These techniques help to identify human responses by using chemicals and biological substances example: In GC-MS, gas chromatography is used to separate complex substance and basic elements from the solution which is further characterized by mass spectra. It is repeatedly done in vitamin and drug research.[9,10]

Analysis of physico-chemical properties of test substances such as pH, absorption spectra, partition co-efficient and other parameter can often indicate potential toxicity. OECD guidelines state that substance with a pH of < 2 or > 11 do not need to be tested in vivo for irritancy potential. Physico-chemical test is fast, quick and cheap and transferable to laboratories easily.[11,12]

2. Computer simulation or Mathematical models:

Mathematical and computer aids are all helpful in the initial stages of research. Simulation means researcher can manage the parameters at will and observe the consequent effects on the model. This way, computer simulation is a beneficial tool for studies and specially for implying new mechanisms or hypotheses to the research work.[13,14] Few classes, the scientists have an idea of using models and unique resources is furnished within the development of simulation software program.[15,16]

The computers stimulation or mathematical model can help to learn the different basic principles of biology. Specialized computer models and software programs which helps to modifying the structure of drug already existed drug molecule and design new molecule. Computer produced simulations model helps to conclude the feasible toxic and biological effects of molecule, drug candidate without animal dissection. The most effective molecules are collected from the initial screening are used for in vivo experimentation. Software's like Structure Activity Relationship (SARs), Computer Aided Drug Design (CADD), Computer Assisted Learning (CAL), etc. is available plenty, with the help of such software programs we can tailor the results and then if necessary in-vivo test can be performed in animals which helps to reduce the total number of animals used in the actual study methodology.[17,18]

Mathematical models are conceptual model that uses mathematical languages, alternative ordinary languages to represent a particular scientific context. Mathematical models help to improve experiment design, conclude the organism's response to varying levels of exposure to a particular chemical. By using the processes of trial and error, a relationship starts to be understood and may be described via mathematical expressions. By collecting research data points, it is easy to fit into mathematical models. Many of the research component may involve kinetic statistics expressed by means of different mathematical equations.[19]

3. Non-invasive imaging (NII) techniques

It is a technique to create photocopy of the human structure for clinical use such as medical procedures find to reveal, diagnose or medical science it includes the work of normal human body. Imaging technology such as the diffusion tensor imaging (DTI), magnetic resonance imaging (MRI), magneto encephalography (MEG), computed tomography (CT) scan, accelerator mass spectroscopy (AMS), nuclear imaging and ultrasound. Above mentioned imaging methods are substituted to make it to irresponsible animal models to give results specific to humans. NII techniques, real-time measurements, very modern and accurate.[39]

4. Molecular docking

Docking studies, mostly conducted in the area of drug design. "It is simply defined as the best or perfect-fit orientation of the ligand molecule to the particular target site result forms a complex". Now a day molecular docking is routinely used to understand the drug-receptor interaction, and it provides the useful information about the binding orientation of drug

molecules so that researcher get knowledge about the affinity and activity of drug molecules.[20,21,22,23]

Docking approaches

In molecular docking community, two approaches are very popular

1. Matching technique
2. Simulates the actual docking

Both approaches have significant advantage and some limitations.

Structure can be determined by some biophysical technique such as nuclear magnetic spectra or x-ray crystallography method. In docking program structure of known protein molecules and potential ligand from data base serve as input. Two things are success of docking program. One is search algorithm and another one is scoring function.[24]

Docking studies and binding free energy calculations of human pancreatic alpha-amylase (HPAA) with embelin and its metal complexes like Copper and Zinc embelin complexes were investigated. Results strongly suggested that the above studied embelin and its metal complexes were potential candidates for human pancreatic alpha-amylase inhibitory activity.[25]

Another study has shown 14 ligands of natural compounds against Human neutrophilelastase (HNE) inhibitors represents a good therapeutic target for the treatment of inflammatory diseases.[26]

5. Micro-array

It is also called DNA/ RNA chips, Bio-chips or Gene-chips. The basic technique of this micro-array is hybridization of nucleic acid. The definition of a micro-array is ordered collection of micro-spots and that each spot consists of a single defined species of nucleic acids based on base pairing rules. In this technique, between two single-stranded of nucleic acid molecules hybridization occurred, it causes sequence complement-tary.[27,28,29] Expression of many gene was quickly concluded by single reaction with efficient manner, this technology helps researchers gain more about various diseases. Microarray expression analysis, Microarray for mutation analysis, Comparative genomic hybridization are the different types of microarray.[30]

6. Pyrogenicity

This experiment was designed for the purpose of to identify the potential bacterial endo-toxin substance present or contaminated to pharmaceutical injectable products. Last twenty years rabbits was used for testing pyrogen in pharmaceutical injectable so that millions of rabbits died. One of the alternative method was developed called pyrogen testing or bacterial endo-toxin test or Limulus Amoebocyte Lysate (LAL) test. The amoebocytes isolated from horseshoe crabs to identify the immune response of pyrogens. Several Invitro models are currently under investigation.[31,32]

Advantages of alternative testing methods:

1. Reduces the usage of number of animals in research.
2. Reduces the animal pain, suffering, and experimental insult.
3. Time saving.
4. More quick result.
5. Reduce the research cost
6. Reduce the error from inter individual species variability.

Disadvantages:

1. No chance to study about the organ (Like histopathology study)
2. No chance to study about metabolic pathway.
3. No chance to recover the organ damaged tissue.
4. Don't have ability to study the organism's growth process.
5. Reduced potential to take a look at the conduct.
6. Decreased capability to examine the interplay among the organism and its surroundings;
7. Reduced ability to study idiosyncratic or species-precise responses.
8. Reduced capacity to differentiate among male and girl-specific phenomena.[1,5]

What are those alternate testing methods or animal substitutes?

LAST CONCLUSION

With the recent advancement of Research and Development, number of animals used for the study has increased enormously. Different methods and alternative organisms are applied to overcome the drawbacks of animal usage, lengthy protocols followed in animal experiments. Moreover, animal models are expensive and they take time to produce results.

In addition to this, Epidemiological surveys, Stem cell research, New imaging technologies, DNA chips will help in reducing use of animals in experiments. Epidemiological surveys are useful to limit the range of investigations regarding a chemical or other substance. Stem cell research extremely beneficial technique for

better understanding of how our bodies work. It reflect a single organ's response and provide better results for study of cancer, liver toxicity, etc. DNA chips are one of the most effective inventions for comparison of thousands of genes at once. New imaging technologies like MRI, fMRI, PET, MRS helps to view the human body and it is not possible by animal experimentation. To overcome the difficulties encountered in animal study, wherever feasible, alternative method can be devised and used. It cannot be possible to eliminate fully animal model but can be reduced in experiments and for research purpose. Various alternative methods need to be implemented in an effective manner.

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VIRAL INFECTIONS OF THE CNS: THE UNRAVELING OF THE MYSTERY

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ABSTRACT

Infections of the central nervous system (CNS) caused by viruses are of public health importance.Viral infections in CNS have always been a challenge to the treating physician. The pathogenesis of viral infection is complex and the determinants include virus, host and environment which are responsible for their varied clinical presentations. The disease manifests differently in immunocompetent and immunosuppressed individuals. Several DNA and RNA viruses are known to have neurotropism with site specificity. Immunogenetics of the host plays an important role in disease outcome. Viral transmission may occur through arthropods, animal bites, human to human contact, oral, vertical and even cannibalism or consumption of raw bush-animal meat. Certain viral infections resolve with neurological sequelae that include cognitive, motor and sensory deficits while certain others are linked with autoimmune diseases. The evolving technologies and advancements in biomedical science will aid in unraveling the mystery behind these viral diseases.

***Key Words**: Acute and Chronic CNS infections, neurotropic viruses, Slow viruses.*

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INTRODUCTION

There are several mysteries in the biosphere. The origin of viruses/prions is a mystery on their own. The theory of origin of viruses include the development of obligate intracellular parasitism from independent microorganism with loss or no biosynthetic capacity, a process called "reductive evolution" of an agent(s). Imagine the Chlamydia-like energy dependent parasite losing its biosynthesis machinery. Mitochondria of mammalian origin could be the progenitor of mammalian viruses. Further, the discovery of giant viruses like Pandoravirus is a mystery on its own. It has been suggested that comets carrying protein building blocks, water and infectious nucleic acid materials could have implicated as influencing evolution of viruses. For the last 40 years, we have known of the ability of self replicating protein infectious material (prions) which strangely is a mutant form of normal cellular protein. Many viruses and prions have the ability to wreak havoc in the central nervous system (CNS) in humans and animals. How and what happens in this process is the mystery and this is at several levels.[1]

The interactive triangle of pathogenesis consists of the three main participants in the malady; the virus, host and environment is shown in Fig.1. Certain viruses of the DNA and RNA groups as well as infectious proteins cause neuropathology and neurological diseases. Usually a small proportion of infected humans develop neurological disease (with varying outcomes) and the subsequent mortality. The disease is differently manifested in immunocompetent and immuno suppressed individuals.[2]

The blood-brain barrier (BBB) is highly selective in its permeability and acts as a barrier between blood and the brain. Anatomically BBB is a cellular barrier, formed by tight junctions between the brain endothelial cells. The BBB allows the passage of water, some gases, and lipid-soluble molecules by passive diffusion, as well as the selective transport of molecules such as glucose and amino acids that are crucial to neural function. Astrocytes and pericytes are necessary to create the BBB. A few regions in the brain, including the circumventricular organs, do not have a BBB. In the major ventricle of the brain there is a choroid plexus (CP) through which the normal physiological fluid viz. cerebrospinal fluid (CSF) is filtered out into the ventricles. This CSF circulates in the ventricular space and between the meninges, the brain, brain stem and spinal cord nourishing the CNS areas. Certain infections produce inflammation in the CP which results in increase in inter cellular spaces because of inflammation causing observable abnormal changes, this is when you see inflammatory cells including lymphocytes in the CSF and pathogens. Non bacterial meningitis; aseptic meningitis caused by viruses' results

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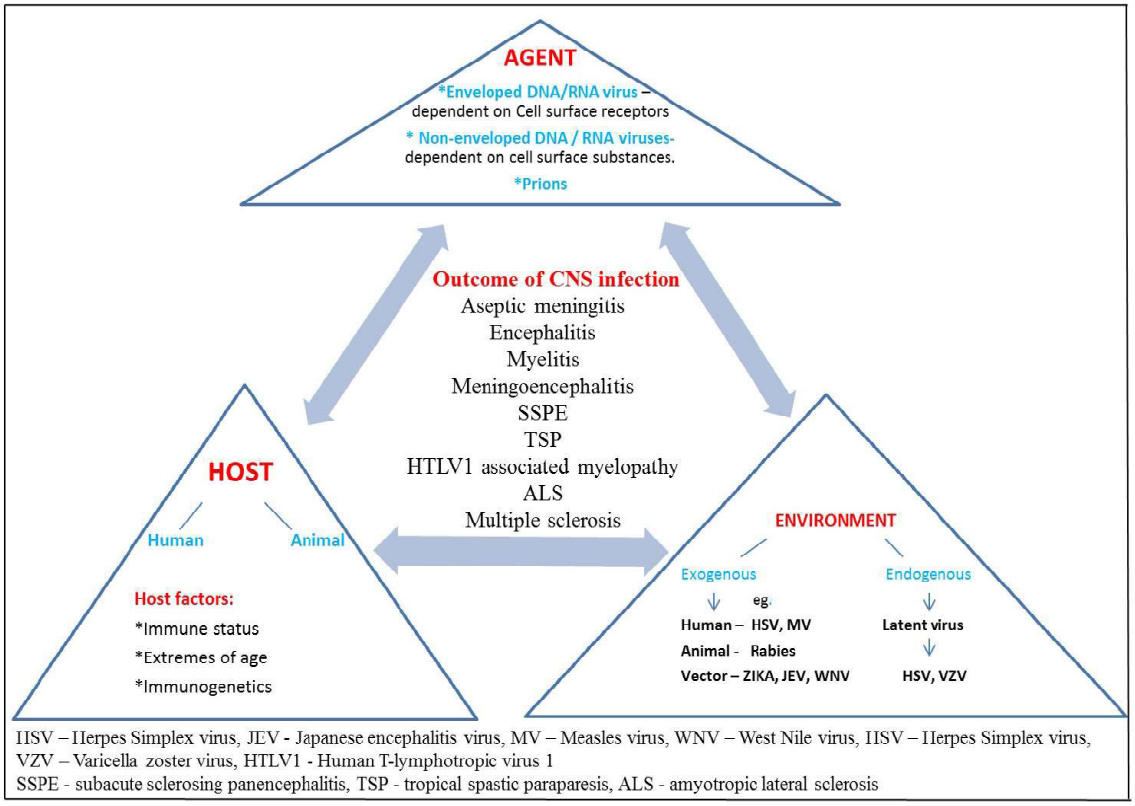


Fig 1. THE INTERACTION OF HOST ENVIRONMENT AND VIRUS IN PATHOGENESIS OF CNS VIRAL INFECTIONS

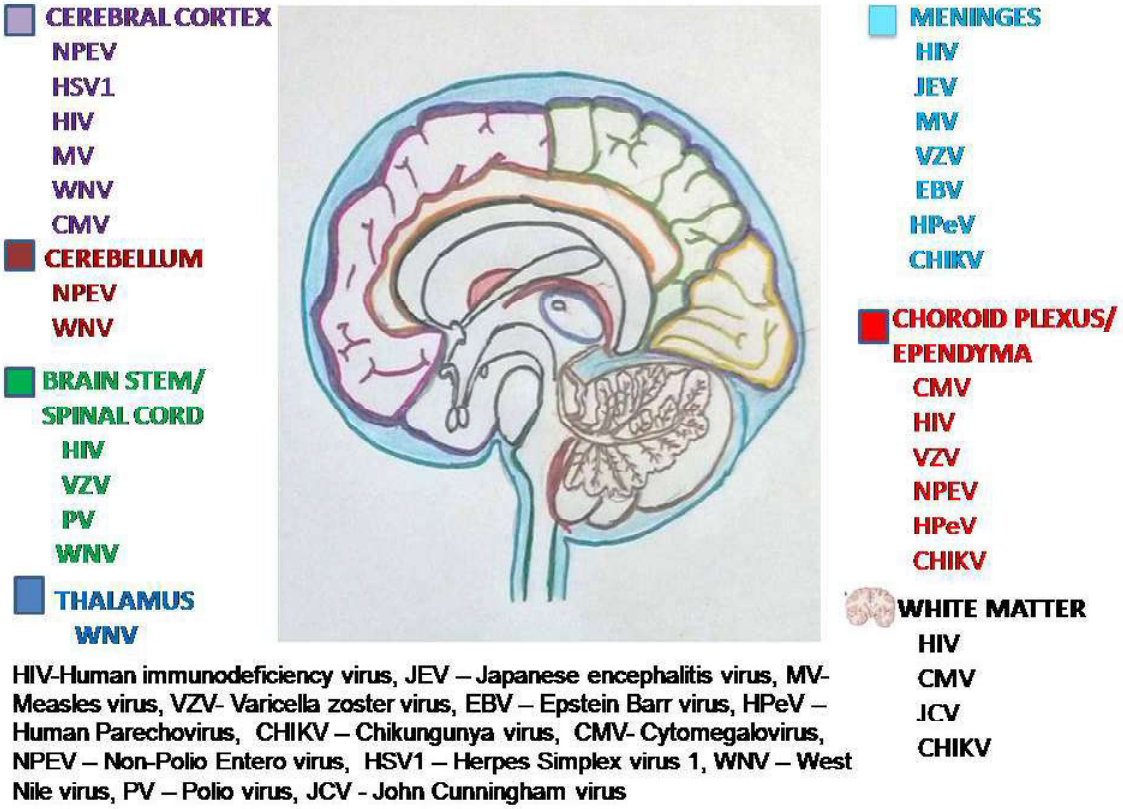


Fig. 2. NEUROTROPISM OF VIRUSES TO DIFFERENT PARTS OF THE BRAIN IN THE COURSE OF AN INFECTION

in increase in lymphocytes. There is specific activation of B and T cells with documentation of intrathecal specific immunoglobulins (antibodies). Only after the resolution of the infections, do CSF parameters return to normal (glucose, protein, cells) it should be emphasized here that the CNS is an immunologically privileged site so is inherently free of inflammatory cells.[3]

VIRAL NEUROTROPISM AND THE HOST

Different viruses with tropism for varying anatomical sites of the brain are shown in Fig.2. Viruses like Human Immunodeficiency Virus (HIV), Japanese Encephalitis Virus (JEV), Measles Virus (MV), Varicella Zoster Virus (VZV), Epstein Barr Virus (EBV), Human Parechovirus (HPeV), and Chikungunya virus (CHIKV) infect meninges; viruses like Cytomegalovirus (CMV), HIV, VZV, Non polio Enterovirus (NPEV), HPeV and CHIKV infect Choroid plexus/ependyma; viruses like JEV, Herpes Simplex Virus -1 (HSV-1), HIV, MV, West Nile Virus (WNV), CMV infect cerebral cortex; viruses like NPEV and WNV infect cerebellum; viruses like HIV, VZV, EBV, Poliovirus (PV), WNV, infect brain stem/spinal cord; viruses like WNV, infect the thalamus; viruses like HIV, CMV, John Cunningham Virus (JCV), CHIKV infect white matter.[4] This association of certain viruses with given sites of the CNS is not rigid. The message here is multiple viruses can infect the CNS at one or more sites. The viruses in particular, neurotropic viruses, use certain cell surface molecule as receptors to which they bind and this is followed by virus penetration of the cell for naked viruses. The enveloped viruses also are dependent on surface receptors for their binding and usually their envelope fuses with the host cell membrane. It is only after this step and internalization of the virus particle does replication begin. Examples of neurotropism of viruses and the receptors used by them are shown in Table-1.

The outcome of CNS infection is variable (Fig.1). Viral infection of CNS leads to complete recovery, partial recovery or fatality. It is important to note that all neurotropic virus infections do not lead to clinical disease, majority may be asymptomatic. Case-in-point poliomyelitis/ encephalitis is seen only in 1:300 of infected individuals of PV/JEV.[5] The interesting feature is polioviruses are transmitted feco-orally and JEV is transmitted by an arthropod vector Culex species, it is to be noted that the disease rate is similar for these two neurotropic viruses despite different routes of transmission. PV can cause any of three manifestations: febrile viral illness, aseptic meningitis or poliomyelitis. Variation in clinical outcome is seen even for one given

Table 1: Determinants of virus-host interactions in neurotropism

Anatomical sites in the CNS infected primarily by virus molecules	Virus	Virus cell surface receptor	Normal cell function of these receptor	Virus antireceptor (on virus particle)
Meninges, cerebral cortex	Measles Virus	CD46	C3b and C4b inactivation	Heamagglutinin
Brain stem/ Spinal cord	Poliovirus (PV1, 2 and 3)	CD155	Establishes tight contact between epithelial cells	Conformationally changed VP1
Cerebral cortex	Herpes Simplex	Heparin sulphate Virus1	Angiogenesis	Glycoprotein B and C
Brain stem/Spinal cord, Thalamus	Rabies virus	Nicotinic acetylcholine receptor (nAchR)	Neuronal impulse transmission at synaptic junction	Glycoprotein G
Brain stem/Spinal cord, cerebral cortex	Human Immunodeficiency Virus 1	CD4	Co-receptor for APC MHC recognition	gp120/gp41
Brain parenchyma (White matter)	John Cunningham virus (JCV)	5-HT_{2A} serotonin receptor	In CNS plays a role in cognition and memory	VP1

Adapted from Becky Schweighardt and Walter J Atwood **Journal of Neuro Virology,** 7: 187± 195, 2001

virus circulating in a given geographical area (topotype). One can conjecture that the host may be hence the determinant of the outcome in this situation.

Outcome of the infection is driven by the host factors; immunocompetence and age of the host. In several viral infections the severity is more at the extremes of age (less than 2 years and over 70 years). The immunogenetics of the host plays an important role both in innate immunity and active (specific) immunity. Toll-like receptors (TLR 3), have been shown in experimental mice to have a protective role against both WNV and JEV infections.[6,7] Pathogen recognition receptors (PRRs) play a significant role as determinants of first line of defense: Toll-like receptors (TLRs) are part of the system. Others that are part of this innate immune system include: retinoic acid-inducible gene I-like receptors (RLRs), the nucleotide oligomerization domain-like receptors (NLRs) and cytosolic DNA sensors (AIM2), intracellular sensors play a role in the clearance of viruses that replicate in the cytosol of cells.[8] Mutations of certain host cell surface molecules can affect the ability of the virus to infect cells. HIV is known to use both CD4 molecules as receptors and to efficiently infect cells another co-receptor the CCR5 molecule is used. Europeans who have a deletion mutation of the cell receptor (CCR5-delta 32) are refractory to HIV-1 infection.[9]

Human Leukocyte Antigen (HLA) haplotypes of host have an important role to play in specific immune response to viruses. Viral T cell antigenic epitopes are presented to CD4+ T cells in the binding groove of Class I major histocompatibility complex (MHC) molecules on non professional antigen presenting cells (APC), almost any infected cell, e.g. tissue fibroblasts including professional APC like macrophages and dendritic cells important for initiating an afferent T cell response generating Cytotoxic T cells (CTL) and memory T cells. Viral epitopes that elicit antibody response are the B cell epitopes which are presented on the professional APC in grooves of MHC Class II antigens with CD4 helper T cell function generating antibody producing plasma cells and memory B cells. Best responses are seen with the epitopes that have high affinity to the host MHC molecules on APC.[10]

Most cytokines are polypeptide messenger molecules (8-140kDa), and some may also be glycosylated. Their biological function in the context of inflammatory response can be differentiated into four major groups: innate immunity (IL-1, IL-5, IL-6, and IL-8); management of inflammatory processes (IL-1, IL-4, and TGF-ß); lymphocyte activation and proliferation (IL-2 and IL-4); and leukocyte growth mediation (IL-1, IL-3, IL-5, and IL-6).

If cytokines are involved in chemical attraction of cells they are referred to as chemokines that can further be split into four major subgroups (CXC, CC, XC, and CX3C) depending on their structural organization of conserved cysteine residues in the amino terminus. A distinct profile of cytokines and chemokines leads to an effective and specific host defense and promotes leukocyte migration during viral CNS infection. During meningitis and encephalitis, an array of cytokines and chemokines has been demonstrated to be regulated including CCL2, CXCL10, CXCL12, IL-1â and TNF-á.

THE VIRUSES

Certain viruses or protein infectious particle (prions) exhibit the attribute of neurotropism. Mutation in RNA genome of polioviruses is associated with loss of neurovirulence, changes in nucleotide (nt) sequence of PV1 genome (57nt), PV2 (2nt) and 10nt changes in PV3 are associated with this phenomenon. Likewise, reversion of attenuated strain to neurovirulant PV2 is known.[11] A high viral RNA in the plasma of HIV infected individuals is associated with symptomatic infection and CNS involvement.[12] Similarly, this association has been observed in plasma virus load and CNS disease for WNV infection.[13] Several researchers have shown the detection of multiple viruses in CNS infections especially members of family *Herpesviridae* both in immunocompetent and immunosuppressed individuals. It is difficult to ascribe aetiopathological significance to the presence of more than one virus in the CSF. It could be postulated that one agent was the primary pathogen and the presence of others could be due to reactivation.[14,15]

Another feature of certain CNS infecting viruses, especially members of family *Herpesviridae* is their ability to establish latency in the host. Some like HSV and VZV do so in the neuronal cells of the sensory nerves ganglions. The protein kinase (66-pk) of VZV gene 66 phosphorylates IE62 and is a gene of VZV latency. This results in accumulation of IE62 in the cytoplasm and reduces nuclear IE62-induced gene activation and thus keeps the virus quiescent. Using the techniques of reverse transcriptase-dependent nested PCR, DNA sequence analysis, in situ hybridization, and immunohistochemistry researchers have revealed VZV open reading frame 66 in latently infected human trigeminal ganglia associated VZV latency.[16]

The cellular activity of epigenetic regulation is used by EBV for its different phases of existence in the host cells. EBV exhibits prelatent, latent and lytic phases in the life cycle. By a unique at each phase a certain set of active and silenced viral genes are seen. Epigenetic modifications of viral gene expression are documentable. The transcription factor BZLF1 is important for the switch from latency to the lytic phase. BZLF1 binds to methylated viral promoters and causes epigenetically suppressed genes to be expressed.[17]

THE ENVIRONMENT

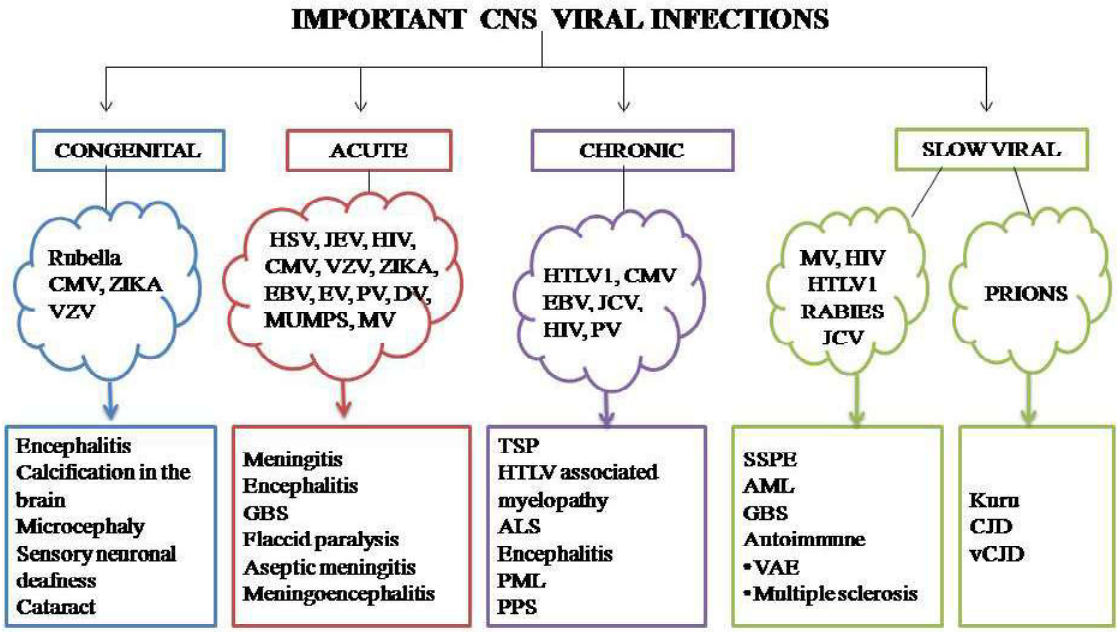
Exogenously acquired animal and human viruses cause disease in humans. Endogenous latent viruses cause disease in immunocompromised host. Many viruses exhibit vector dependence to reach humans. There are differences in tropical, temperate and cold climates in terms of circulation of certain viruses. Competence of vectors and transmission of neurovirulent viruses is an issue to be well understood especially with the emerging threat of Zika virus which is now seen in North and South America as well as in East Asia. Ecological niches of these agents vary. Some are transmitted via arthropods, some by animal bites, human to human contact, orally, vertically and even cannibalism or consumption of raw bush-animal meat (HIV transmission to humans). The emerging infections result from destruction of forests and subsequent interaction between sylvatic and urban cycles of certain viruses.[18] Acquisition of certain viruses is shown in Table-2.

IMPORTANT VIRAL INFECTIONS

Important viral infections of CNS, causative agent and its manifestations are shown in Fig.3.

Congenital viral infections: Several viruses are known to be associated with congenital infections of the new born with CNS involvement typically this includes maternal infections transmitted to the fetus by the transplacental route or during delivery, infection happening in the birth canal. Maternal infection with Rubella, CMV, Zika early in pregnancy (i.e fetal morphogenesis) is associated with severe CNS involvement of the new born. The manifestations may be encephalitis, calcification in the brain, cataract, microcephaly or sensory neural deafness.[19]

Acute CNS infections: Several acute CNS infections manifest with changes in cognitive function, signs of depression, acute motor and sensory changes which will soon be followed within 72 hours of manifestations of CNS disease with classical changes in the biochemistry and cellular features of the CSF indicative of viral etiology. These include infections with HSV, JEV, HIV, and CMV.[20] In the case of JEV the virus reaches the CNS by viremic spread or through



HIV-Human immunodeficiency virus, JEV – Japanese encephalitis virus, MV- Measles virus, VZV- Varicella zoster virus, EBV – Epstein Barr virus, CHIKV – Chikungunya virus, CMV- Cytomegalovirus, HSV – Herpes Simplex virus, WNV – West Nile virus, PV – Polio virus , HTLV1 – Human T-lymphotropic virus 1, DV- Dengue virus, JCV – John Cunningham virus, EV – Entero virus. GBS- Guillain-Barre Syndrome, TSP- tropical spastic paraparesis, ALS – amyotropic lateral sclerosis, PML- progressive multifocal leukoencephalopathy, PPS – post-polio syndrome, SSPE – subacute sclerosing panencephalitis, VAE – vaccination allergic encephalomyelitis, CJD – Creutzfeldt-Jacob disease, vCJD – variant CJD

Fig.3. Important Viral infections of central nervous system

Table-2: Important neurotropic viruses: Their geographical distribution, transmission and their potential to cause congenital infections

Virus	Geographical distribution	Transmission	Potential for congenital infections	CNS manifestations	Nature of spread
HSV	Global	Close personal contact, sexual, respiratory and genital routes	Perinatal infections are known for HSV2/ HSV1	Encephalitis, meningoencephalitis	Sporadic
VZV	Global	Close personal contact, respiratory route	Perinatal infections are known	Encephalitis, meningoencephalitis	Sporadic
Rubella	South Asia, Africa	Close personal contact, respiratory route	Congenital infections are known	Encephalitis, meningoencephalitis, retinitis, sensory neural deafness, mental retardation	Sporadic/ Outbreak
CMV	Global	Close personal contact, respiratory route and vertical transmission	Congenital infections are known	Encephalitis, ventriculoencephalitis, transverse myelitis, polyradiculomyelitis	Sporadic
Zika virus	S.America, N.America, S. E. Asia	Mosquito *Aedes spp,* vertical and sexual	Congenital infections	Brain ischemia, myelitis, meningoencephalitis	Epidemic
WNV	N.America	Mosquito *Aedes spp, Culex spp*	Nil	Meningoencephalitis, encephalitis, myelitis	Epidemic
JEV	Asia	Mosquito *Culex Spp*	Nil	Encephalitis, meningoencephalitis	Outbreak/ Epidemic
Non-polio enterovirus	Global encephalitis	Feco-oral route	Neonatal sepsis	Polio-like illness,	Outbreak/ Epidemic
HIV	Global	Horizontal, vertical, and iatrogenic	Perinatal transmission	GBS, polyradiculapathy, myopathy, focal neuropathy, Chronic encephalitis-like syndrome with PML	Pandemic/ Outbreak

the olfactory nerves of the cribriform plate of ethmoid like influenza virus.[21] Another form of viral spread to the CNS and subsequent pathology is seen with VZV. The virus spreads from skin/mucosa into sensory nerve endings. Virus travels to dorsal root ganglion (DRG) and becomes latent. Reactivation occurs with decreased cell-mediated immunity. Initial replication occurs in affected DRG after reactivation.[22]

Spread of Rabies virus to CNS may take a few weeks to 18 months. It depends on the amount of virus contained in the saliva which is deposited at the site of the bite of an infected animal. It is a fatal disease if post exposure prophylaxis is not given within 7 days of the bite, if the bite is in the limbs. Facial bites by infected animals may lead to rapid development of the disease.[23]

Retroviral infections of the CNS can occur as an acute or chronic condition. In the case of HIV, Guillain-Barré syndrome (GBS) could be seen as part of acute sero-conversion illness. The virus easily crosses the BBB through cell to cell spread or seeding in the CSF through infected CD4 cells. HIV causes CNS manifestations

but this is usually seen with opportunistic pathogens when the individual is immunosuppressed in the course of HIV disease. The virus indirectly destroys cells in the CNS and also causes sustained CNS inflammation, accelerated vascular disease with amyloid deposition.[24]

The Enterovirus genus includes over 100 serotypes/ genotypes. At least 20 are known to cause CNS infections. The conditions range from acute flaccid paralysis (AFP) to polio-like-illness. AFP caused by NPEV generally improves to restoration of motor functions unlike permanent sequelae with infections of polioviruses. Both PV and NPEV enter through the mouth, replicate in the pharynx, GI tract and local lymphatics. From here there is a hematogenic spread to the CNS. Viral spread along nerve fibers could cause destruction of motor neurons .[25]

GBS is post infectious sequelae marked by demyelination which could be localized or ascending. An acute inflammatory demyelinating polyradiculo neuropathy (AIDP) with acute motor-sensory axonal changes is a subtype of GBS. [26] The viruses associated include EBV, VZV, CMV, Dengue viruses, Zika virus. Systemically and locally released pro-inflammatory cytokines (IL-1ß, TNF, IL-6) are responsible for demyelination.

Chronic CNS infection other than slow viral diseases: HTLV-1 is usually associated with adult T cell leukemia but has been implicated in certain neurological conditions like tropical spastic paraparesis (TSP)/ human T-lymphotropic virus (HTLV-1) associated myelopathy (HAM), amyotropic lateral sclerosis.[27] In addition CMV and EBV are known to establish chronic encephalitis in immunosuppressed patients. EBV is associated with chronic fatigue syndrome in the Northern hemisphere. Here the virus is known to transiently appear in the CSF compartment of the CNS.[28]

Progressive Multifocal Leukoencephalopathy (PML) is a condition caused by John Cunningham Virus (JCV). Here there is asymmetric involvement brainstem and is seen more commonly in AIDS patients. In non HIV infected patients who have T-cell immunodeficiency, lymphoproliferative disorders chronic granulomatous diseases, solid organ transplant recipients or hematologic malignancy PML could also be seen.[29]

Post polio syndrome (PPS) is seen several years after polio affliction. A possible mechanism for PPS is motor neuronal loss due to reactivation of a persistent latent virus. Muscle atrophy and denervation is seen, foci of perivascular and interstitial inflammatory cells have been found in 50% of biopsies of patients with PPS. Activated T cells and immunoglobulin M and immunoglobulin G antibodies specific for gangliosides also have been found. Another possibility is an infection of the polio survivor's motor neurons by another enterovirus (Acute Flaccid paralysis agent) that is different from the one responsible for the patients' polio condition.[30]

SLOW VIRAL INFECTIONS

SSPE: Measles is an acute febrile exanthematous condition that is usually a self-limiting disease, but it can be associated with several complications, one of which is subacute sclerosing panencephalitis (SSPE) which manifests several years later. Rapid replication of MV that has been quiescent for years is triggered by some reactivation event(s) and results in hyper-reactive immune responses. Demyelination in persistent MV infections is due to a complex combination of viral cytopathic effects on neuronal cells and immune-mediated mechanisms. The pathogenesis of persistent MV infection in SSPE is not very clear.

Prions in CNS disease: These agents cause variety of human and animal disease. Certain form of animal prion disease is transmissible to humans. The pathogenic prion protein is a mutant form of a normally expressed protein in low amounts in the host cells. The protein is very stable to moist heat. The normally expressed protein is different from the prion protein seen in disease. Certain features of slow viruses' and prion diseases are shown in Table-3. Table-4 shows differences between the normal cellular prion protein and the pathological prion protein. Variant Creutzfeldt-Jakob disease (vCJD) is a rare degenerative fatal brain disorder. It was reported in the year 1995. It was believed to be due to ingestion of beef products contaminated by prion agent a variant of bovine spongiform encephalitis producing agent. Early psychiatric symptoms and sensory symptoms are much more common here and cerebellar findings are present in all patients with vCJD.[31]

Amyotrophic lateral sclerosis (ALS): A progressive, invariably fatal neurologic disorder resulting from upper and lower motor neuron degeneration. This condition typically develops during the sixth or seventh decade of life, and is diagnosed based on standard clinical criteria. A small percentage of persons infected with the HIV-1 or HTLV-1 develop ALS-like syndromes. While HTLV-1 associated ALS-like syndrome has several features that may distinguish it

Table 3: Slow viral and Prion infections of Humans

Disease	Agent	Host(s)	Incubation period	Nature of Disease
SSPE	MV	Humans	2-20yrs	Chronic sclerosing panencephalitis
PML	JCV	Humans	Years	CNS demyelination
PRPE	Rubella virus	Humans	Years	Chronic encephalitis
Kuru	Prion	Humans	Months to years	Spongiform encephalopathy
Creutzfeldt-Jacob disease (CJD)	Prion	Humans	Months to years	Spongiform encephalopathy
vCJD	Prion	Bovine, Humans	Months to years	Encephalitis, myelitis

Table-4: Differences between cellular and scrapie proteins

Attribute	PrP^{c}	PrP^{sc}
Solubility	Soluble	Non soluble
Structure	Alpha-helical	Beta-Sheeted
Multimerisation state	Monomeric	Multimeric
Infectivity	Non-infectious	Infectious
Suceptibility to proteinase K	Susceptible	Resistant

PrP^{c}: normal cellular expressed prion protein (low level)

PrP^{sc}: pathological protein expressed in disease- scrapie protein (high level)

from classical ALS, HIV-infected patients may develop neurological manifestations that resemble classical ALS although it occurs at a younger age and they may show a dramatic improvement following the initiation of antiretroviral therapy.[32]

Virus infections as a trigger of Autoimmune Disease: Natural infections can cause exacerbations of autoimmune disease. This is most likely due to the induction of IL-12, IL-6 and IFN-ã. Previously, when nerve tissue derived rabies vaccine was used before the advent of wide usage of cell culture based vaccine post vaccination allergic encephalomyelitis was reported at a frequency of 1 in 5000 vaccines. The risk was higher when individuals received repeat cycles of post exposure prophylaxis. This had been attributed to immune response triggered to myelin basic protein, an autoimmune phenomenon. Normally the CNS is an immunologically privileged site; hence, myelin basic protein is not recognized as a self-antigen.[33]

Another autoimmune-like disease condition is multiple sclerosis (MS) which is characterized by demyelination of nerve cells in the brain and spinal cord. Certain viruses have been linked to this process. MVwas speculated to be involved and there is some evidence for linking JCV with this condition.[34]

CONCLUSIONS

Infections of the CNS caused by viruses are of public health importance. They are acute or chronic in nature. The viruses that cause acute infection have a pathophysiology different from those that cause chronic or slow infections of the CNS. Protein infectious agents (prions) cause slow virus disease of the brain and have several unique features which distinguish them from viruses that contain genomic material which is RNA or DNA in nature. The viruses and prions reach the CNS either by breaching the BBB or traversing in the axonal sheath. Very often, several viruses have a strong viremic phase before CNS invasion. Innate and specific immunity of the host determine the establishment of the viral infection, CNS invasion and clinical outcome. Viral attributes, host immunogenetics and certain environmental factors determine the distribution and disease causation of viruses. Viral infection of the CNS could have acute or chronic morbidities which quite often result in fatality with a few exceptions. Poliomyelitis is a clinical condition with low fatality rate of all CNS infections but the sequelae of paralyzed limb(s) persist without harming cognitive function. Certain viral infections resolve with neurological sequelae that include cognitive, motor and sensory deficits. Several unknown features exist in the understanding of the pathophysiology of CNS viral infections.

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MANAGEMENT OF POSTBURN LYMPHEDEMA BY Z-PLASTY

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ABSTRACT

Introduction: *Circumferential lower limb scarring acts like a ring constriction and produces distal lymphedema.*

Case: *A 35 yr old lady presented with left lower limb edema of 5yrs duration secondary to burn wound infection. She was managed with manual massage, graduated compression stockings, wound care and Z-plasty.*

Result: *Lymphedema reduced and symptoms improved.*

Conclusion: *The skin burn wound has to be treated early and appropriately to minimize complications.*

Key words*: Constriction ring, Lymphedema, Z-plasty.*

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INTRODUCTION

The management of burn wound needs to be done with utmost care to prevent complications. Janzekovic procedure of tangential excision with immediate skin grafting helps to heal the wound faster.[1] The procedure causes considerable blood loss and has to be done with caution. An unusual presentation of post burn skin infection of lower limb which healed by secondary intention and presented as lymphedema of lower limb is discussed. Lymphedema can develop when there is obstruction to its flow. Circumferential lower limb burn scarring impedes lymph flow and can cause distal edema of foot and leg. The scar band needs to be released and flaps need to be fashioned to augment lymphatic flow. The circumferential scarring in this patient acted like a ring constriction syndrome and produced distal lymphedema.

CASE

A 35 yrs old lady complained of left lower limb edema of five years duration. She had sustained flame burn to the area ten years ago. The patient lives in a remote location without access to proper surgical care. The burn wound which was managed conservatively and was complicated by superficial skin and subcutaneous infection. Patient does not give history of repeated lower limb swelling suggestive of filariasis prior to the burn. On examination, there was no active sign of infection. A near circumferential scarring on left lower limb was present. The left leg and foot was swollen and non-pitting. Skin changes like hyperpigmentation and irregular scaring on anterior aspect of lower leg was also present (Fig.1). The other limb was normal. The patient was admitted and leg elevation was done after applying graded compression elastic stockings. The edematous limb was cleaned daily with dilute betadine solution and saline and mupirocin ointment was applied topically. Manual limb massage was given daily for 10 days by our physiotherapist. The limb girth measurement and weight of the patient was recorded daily. The limb girth reduced by five inches in ten days (Fig.2). The patient's weight reduced by 2 kg and remained the same till discharge. The reduction in limb girth helped in making the skin supple. The patient was operated upon and the scar band was excised in toto. Multiple small Z-plasty was done to augment lymphatic flow. The skin below the constriction band became supple and swelling of the leg and foot reduced (Fig.3). The graded compression dressing and foot end elevation was continued. There was further reduction by two inches in limb girth on tenth postoperative day. Patient was discharged with compression stockings.

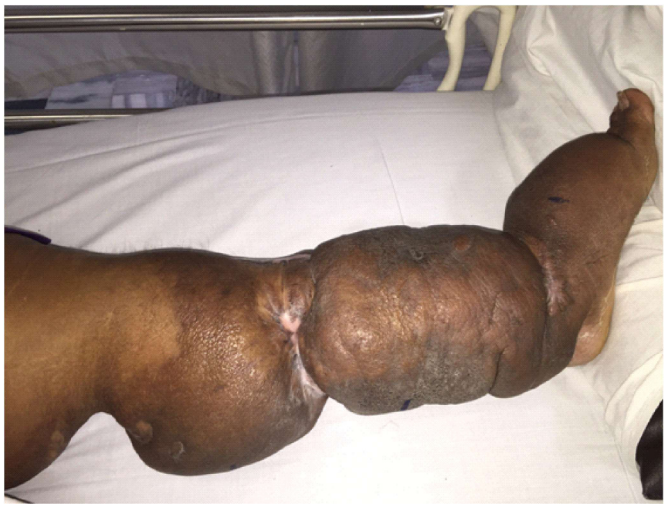

Fig.1: Left lower limb at the time of admission

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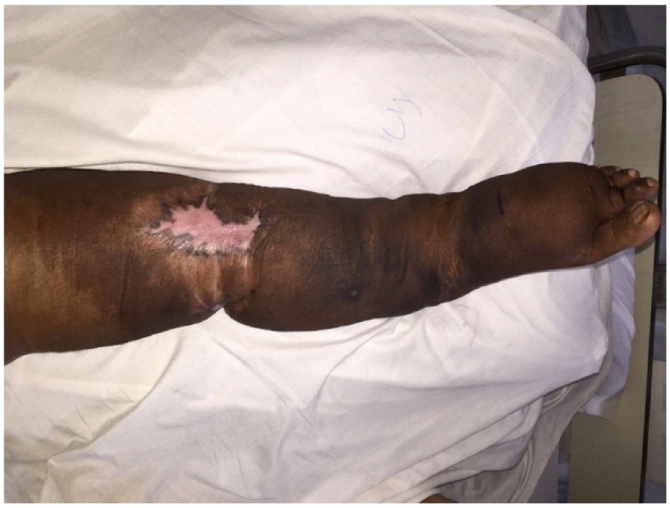

Fig.2: Left lower limb after Manual massage

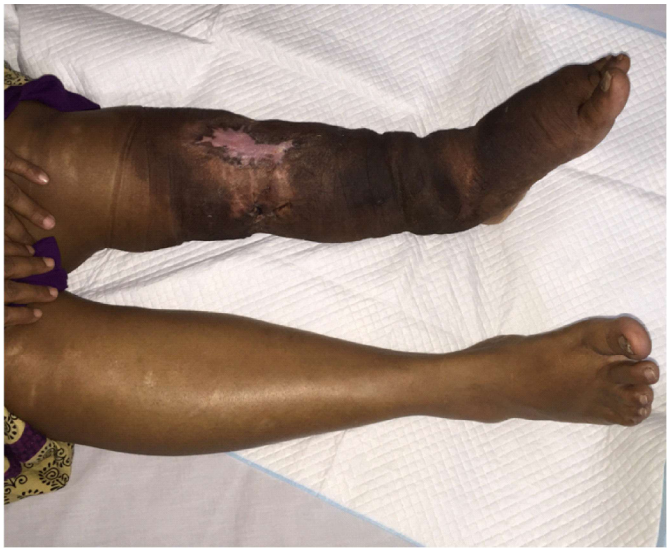

Fig.3: 14 days post op - after Z plasty

DISCUSSION

Lymphedema is a chronic condition and difficult to treat.The reason for development of edema is imbalance between demand and capacity of lymphatic circulation. The progressive obstruction of the lymphatic system produces interstitial fluid rich in protein. This causes inflammation, adipose tissue hypertrophy and fibrosis. The swelling of affected region can cause deformation, decreased mobility and function. Lymphedema can occur primary or secondary to malignancy, radiation, block dissection and infection.[2,3,4]

The proposed clinical classification of lymphedema by International Society of Lymphology[5] is

- Stage 0: Sub clinical infection where swelling is not evident even though impaired lymph transport.
- Stage I: Limb edema subsides on elevation. Pitting present
- Stage II: Limb elevation rarely reduces edema. Pitting present or absent
- Stage III: Pitting absent. Trophic skin changes such as warty over growths, acanthosis, hyperkeratosis, papillomatosis and skin ulcer can occur.

The case described was in Stage III as described by the above classification.

Classical signs in lymphedema of lower limb on physical examinations are

(i) Peau d' orange changes of the skin (because of cutaneous and subcutaneous fibrosis).

(ii) Positive Stemmer sign (inability to grasp the skin of the dorsum of the second digit of the feet)

Lymphoscintigraphy is considered the gold standard for diagnosing lymphedema.[6] The investigation is done by injecting radio labeled colloid intradermally in distal edematous limb and then imaging lymphatic vasculature. The study provides lymphatic anatomy and its function. Typical abnormalities seen are absent or delayed radiotracer transport, cutaneous flare, dermal diffusion or back flow and poorly visualized lymph nodes.MRI is useful in diagnosis of lymphedema without radiation exposure. Classical signs seen on MRI are skin thickening, "Honey combing" of subcutaneous tissue, epifascial fluid lakes and absence of edema with in muscular compartments.

The current international standard of care for lymphedema is formalized in a 2013 consensus document of the International Society of Lymphology. Treatment of lymphedema is by decongestive therapy in two-phase approach. The first phase is reduction of swelling which involves proper skin care (cleansing, low pH lotions, emollients), manual massage to drain lymph, range of motion exercises and compression with multilayered bandages. The second phase is the long-term maintenance of volume, which includes skin care, regular exercises, graduated compression stockings, limb elevation and pneumatic lymph drainage. A graduated compression stocking with highest level of compression (20-60 mmHg) that the patient can tolerate is most beneficial.However, lower compression can be used for milder lymphedema or general leg edema.[7,8]

Decongestive treatment would increase lymph flow, augment lymphatic contractility and reduce

extremity lymph fluid. Benzopyrene is a commonly used drug which acts by increasing proteolysis by the macrophages. The use of the drug is controversial as long-term use of benzopyrene is hepatotoxic. Another commonly used drug is diuretics. Diuretics produce immediate relief by removing intravascular or interstitial fluid but this can be deleterious by producing increased fibrosis due to protein accumulation.

For patient not responding to decongestive therapy, surgery is an option. Surgical options available are excisional approach, suction assessed lipectomy and microsurgical lymphatic anastomosis. For circumferential contracture lymphedema, surgical approaches are Z plasty, rectangular flaps and simple excisional surgery.[9,10,11]

CONCLUSION

Burns need to be treated in tertiary centers for proper healing. The skin burn wound has to be treated early and appropriately to minimize complications. Circumferential full thickness limb burn can produce circumferential contracture. The circumferential contracture can disturb lymphatic drainage producing distal edema. Presence of unburned bridge of skin on limb may help to avoid distal lymphedema. Early proper management of burns could preserve the bridge of skin. If burn is circumferential without bridge of skin, then the bridge may be recreated by plastic surgical principles.

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ROLE OF GROSS PATHOLOGY IN OVARIAN CYSTIC LESIONS: GROSS AND MICROSCOPIC PATHOLOGY OF THREE OVARIAN CYSTIC LESIONS

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ABSTRACT

Gross pathology helps in framing a presumptive diagnosis in the mind of surgical pathologist. Sometimes, microscopic examination differs from the presumptive diagnosis framed during gross pathology examination. Three ovarian cystic lesions which were given a presumptive diagnosis of serous cystadenocarcinoma, borderline serous cystadenoma, mucinous cystadenoma in gross pathology examination were reported as well differentiated endometrioid carcinoma, juvenile granulosa cell tumour and atypical proliferative sero-mucinous cystadenoma.

Keywords*- gross examination, endometrioid carcinoma, sero-mucinous tumour, granulosa cell tumour*

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INTRODUCTION

Gross pathology is the recognition, description of abnormalities and pathologies at the clinical level. Gross pathology aids in suggesting a pathogenesis, mechanism of clinical disease, and establishing a presumptive diagnosis. In addition gross pathology, aids in selection of relevant portions for microscopic examination and special studies. Diagnosis on the basis of gross pathology can be made in around 90% of specimens. In the remaining 10% of specimens, a differential diagnosis can be arrived at before microscopic examination.[1,2] On that accord, we present our experience in correlation of gross examination with histopathological diagnosis in ovarian cystic lesions. Three ovarian cystic lesions wherein the histopathological diagnosis differed from presumptive diagnosis established at gross examination are also highlighted.

MATERIALS AND METHODS

A retrospective analysis was done by reviewing the surgical gross and microscopic examination reports of patients who underwent surgical management for ovarian cystic lesions from June 2015 until June 2016. The presumptive diagnosis was classified into non-neoplastic and neoplastic ovarian cystic lesion. The presumptive diagnosis obtained from gross examination was compared with the final histopathological diagnosis. The histopathological diagnosis was categorized into non-neoplastic and neoplastic ovarian cysts. The neoplastic ovarian cysts were further categorized according to WHO classification. Descriptive statistics were used to determine between gross diagnosis and histopathological diagnosis.

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RESULTS

Forty eight ovarian cystic lesions were surgically managed. On gross examination, 26 were classified as non-neoplastic ovarian cysts and 22 were classified as neoplastic cystic lesions. On histopathological diagnosis, all the non-neoplastic presumptive gross diagnosis correlated with the final microscopic examination. Out of 22 neoplastic cystic lesions, good gross-histopathological correlation was observed in 19 cases and 3 cases (13.63%) were not correlated. All the three cases though were termed neoplastic in gross examination; the presumptive diagnosis and histopatholgical categorization of ovarian cystic lesion were not correlated. The non-correlated cases are described below;

CASE-1

A cystic mass measuring 9 x 6 x 3.5 cms was received in formalin. Cut section the cyst appears multi-locular, with cystic and solid friable areas with papillary excrescences (Fig 1). A presumptive diagnosis of serous carcinoma was made. On microscopy, infiltrating back to back glands, with few areas showing

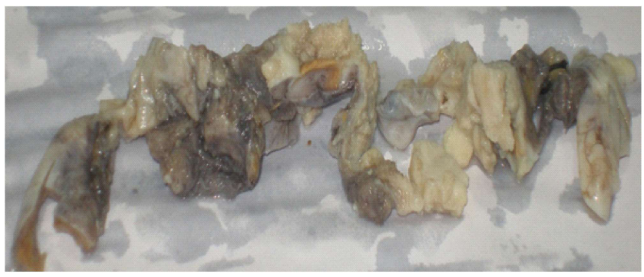

Fig 1: Multi-locular cyst with solid friable areas and papillary excrescences

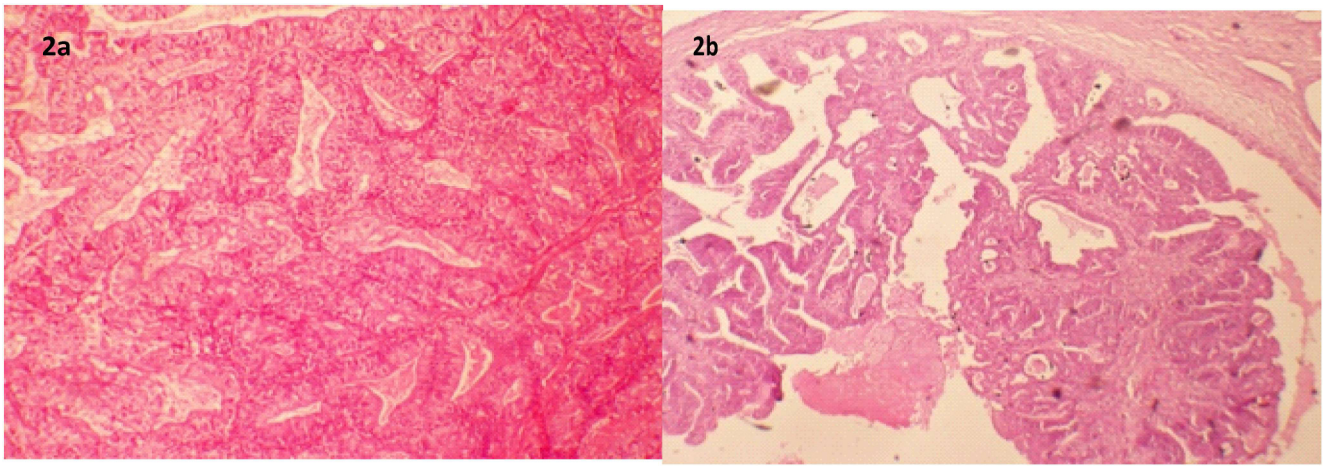

Fig 2a: Infiltrating confluent glandular pattern lined by stratified columnar cells (Haematoxylin and eosin, 10x)(H&Ex100); 2b: Cyst wall with papillary excrescences lined by glands with stratified columnar cells (Haematoxylin and eosin, 10x) (H&Ex100).

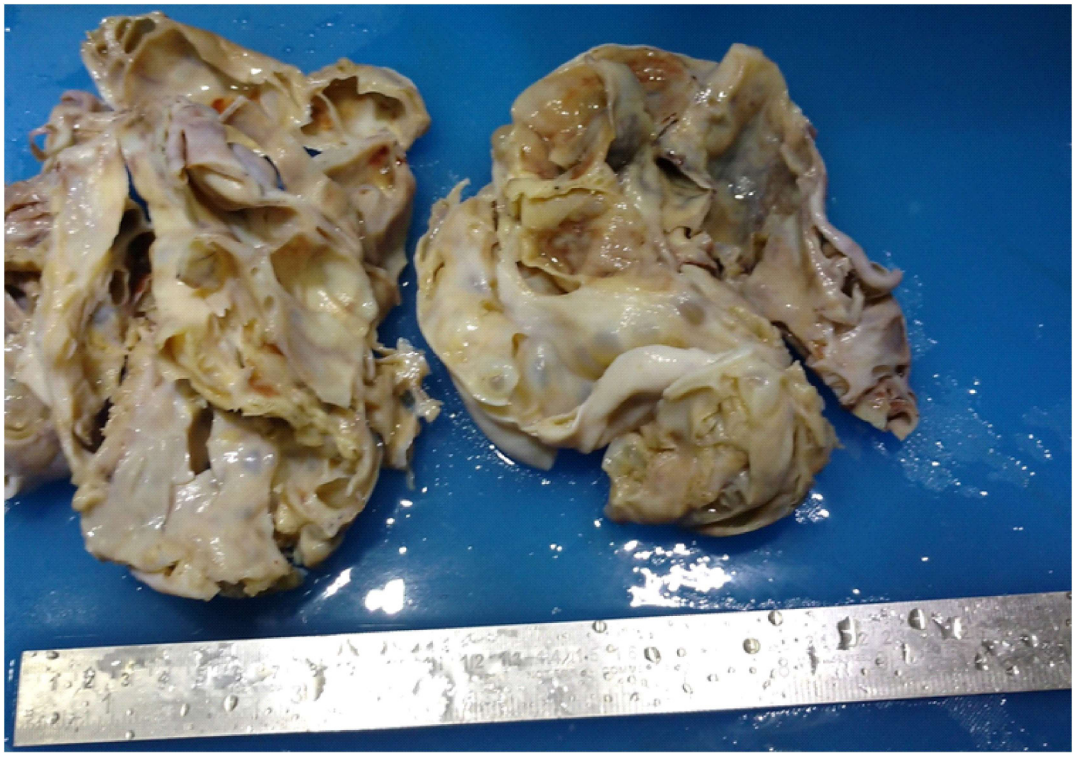

Fig 3: Multi-locular cyst with thickened cyst wall (H&Ex100).

papillary configuration and characterized by stratified columnar epithelial cells with focal squamous differentiation was identified (Fig 2 a, b). A diagnosis of well differentiated endometrioid carcinoma was made.

CASE-2

A cystic mass measuring 20 x 16 x 9 cms was received in formalin. Cut section the cyst appears multi-locular, filled with clear straw coloured fluid with thickened cyst walls (Fig 3). No solid areas were identified. A presumptive diagnosis of borderline serous cystadenoma was made. On microscopy, cyst wall lined by stratified nests and macro-follicles of round cells were seen. The cells were characterized by hyperchromatic nuclei with rare nuclear grooves and increased mitosis (Fig.4a,b). Immunohistochemistry of Epithelial Membrane Antigen (EMA) revealed a negative staining. A diagnosis of juvenile granulosa cell tumour was made.

CASE-3

A cystic mass measuring 26 x 23 x 16 cms was received in formalin. On cut section, a multi-loculated

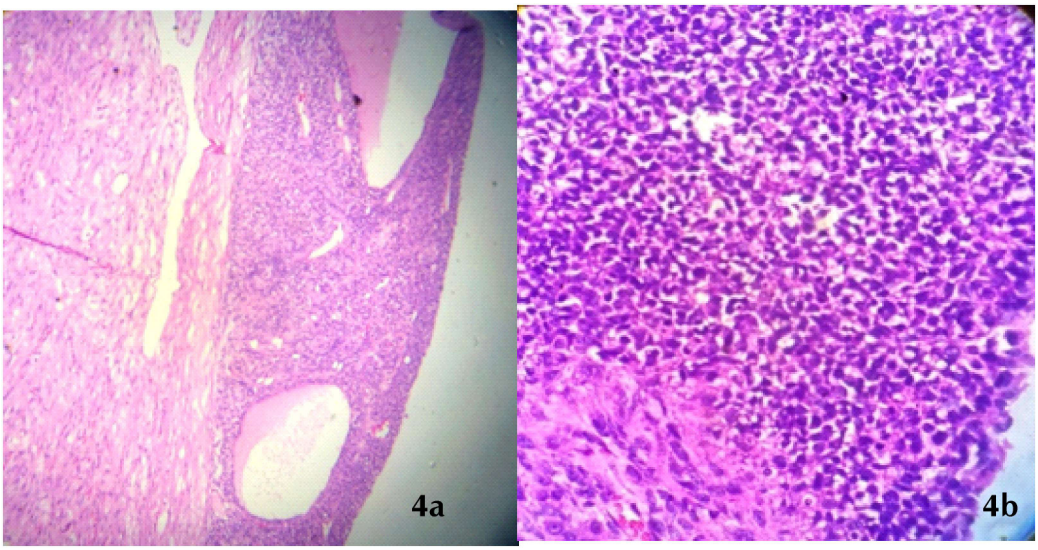

Fig 4a: Cyst wall lined by multi-layered round cells with hyperchromatic nuclei with focal follicle formation (Haematoxylin and eosin, 10x) (H&Ex100);

Fig 4b: Hyperchromatic cells with increased mitosis (Haematoxylin and eosin, 40x) (H&Ex100)

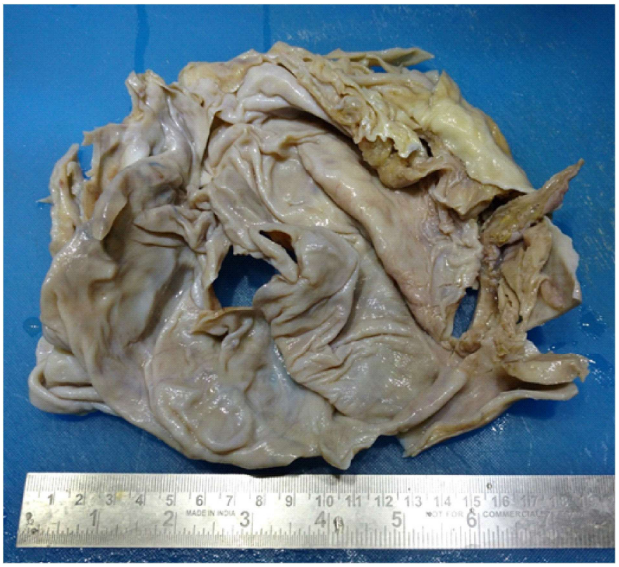

Fig 5: Multi-locular cyst with smooth walls

cyst filled with sero-mucinous fluid was expressed. No solid areas were identified (Fig 5). A presumptive diagnosis of mucinous cystadenoma was made. On microscopy, cyst wall lined partly by columnar mucinous cells and partly by cuboidal cells with focal stratification and atypia in approximately 10 to 12% of tumour was identified (Fig 6 a, b). A diagnosis of atypical proliferative mucinous tumour of sero-mucinous or mullerian type was made.

DISCUSSION

The attributes of gross pathology includes distribution, contour, shape, colour, size, consistency, special features and extent .[2] The gross lesions of some pathology entities are sufficiently distinct in their pattern to be presumptively diagnostic. For other entities, a differential diagnosis can be made before confirmation with microscopic examination. Sometimes, there is discordance in the presumptive diagnosis established by gross examination and microscopic examination.[1-3] In case-1, the solid friable areas and papillary excrescences made us think of serous carcinoma. However, the solid areas and papillary areas were made of confluent back to back arrangement of glands lined by stratified columnar cells. Though papillary excrescences are most commonly seen in serous cystadenocarcinoma, it is also less commonly seen in endometrioid carcinoma.[4] Clinical knowledge and background information of ovarian cysts which can have papillary architecture is instrumental in constructing the differential diagnosis. In case-2, multi-locular cyst with smooth lining and occasional thickened wall is indistinguishable from various other cystic masses. Due to increased prevalence of surface epithelial lesions, and mild thickening of wall, a presumptive diagnosis of borderline serous cystadenoma was considered. The clinical background knowledge of normal levels of CA-125 which was not considered at the time of gross examination would have helped in considering other cystic non surface epithelial tumours.[4,5] In case-3, though the gross examination resembled that of a typical mucinous cystadenoma, sectioning and microscopic examination from various areas as per institutional protocol revealed dual lining of cyst wall. This case demonstrates the importance of sectioning from different areas from a large tumour.[1]

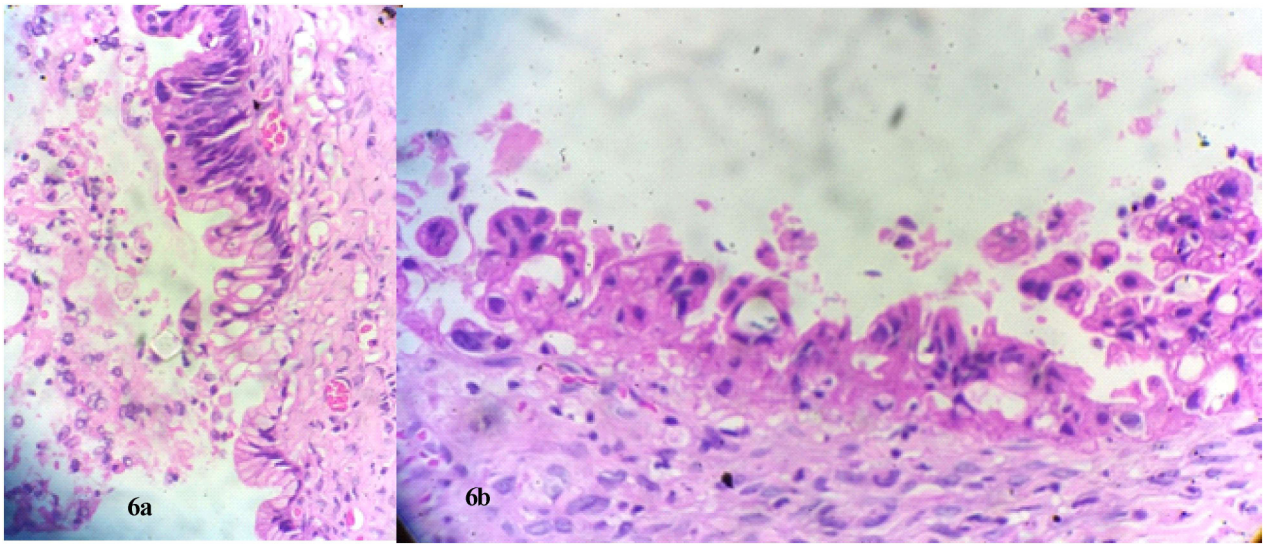

Fig 6a: Cyst wall lined by bland mucinous cells and stratified columnar cells with hyperchromatic nucleus (Haematoxylin and eosin, 10x) (H&Ex100);

Fig 6b: Cyst wall lined by stratified columnar cells, few with hyperchromatic nuclei and atypia (H&Ex400)

Although gross examination is not a diagnostic test on its own, it plays a crucial role in providing a correct histopathological diagnosis.[6] To our knowledge, there are no studies regarding the correlation of gross-histopathology examination. In our study, there was 100% correlation in non-neoplastic ovarian cysts and 86.37% correlation in neoplastic ovarian cystic lesions. Since gross examination is not a diagnostic test on its own, the accuracy, sensitivity and specificity were not determined.

With the advent of molecular pathology, the traditional pathology skill of gross examination is rapidly declining amongst young pathologists. There is combined loss in quality of gross examination, accuracy, elegance of specimen description and lack of specificity in sample selection for microscopic, immunohistochemical examination, molecular studies.[1] With this article, we would like to emphasize the importance and principles of gross examination. The two principles of gross examination are knowledge of clinical history and knowledge of organ or region examined. Also, another prerequisite for gross pathology is consistency in sections taken according to standard manuals or institutional protocols.[1] A microscopic examination from a poorly selected site of specimen can be catastrophic if leads to a wrong diagnosis. Similarly, a special study from improperly selected site will be meaningless.[1,2] As pathology residents are the personnel mostly responsible for gross examination, importance and protocols of gross examination of pathology specimens must be taught as part of pathology education programme.

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IMPORTANCE OF SAMPLE SIZE IN MEDICAL RESEARCH

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It is an important and very crucial aspect in the present day evidence based medical/clinical research study is deciding how big the sample would be. There is a lot of statistical jargon prevailing in calculating the sample size for a given study / study objective. This first piece of series of statistical notes discusses about basics of sample size for certain type medical research study, also demystifies the 'difficult' statistical terminology involved in sample size calculation.

If a study increases the sample size there will an increasing precision in the study estimates for which one doing a study. Which means for any given estimate, the greater the sample size the more "statistically significant" the result will be. In other words, if an investigation is too small (sample size small) then it will not detect resultsthat are in fact important. Conversely, if a very large sample is used, even tiny deviations fromthe null hypothesis will be statistically significant, even if these are not, in fact, practicallyimportant. In practice, this means that before carrying out any investigation you should have an idea of what kind of change from the null hypothesis would be regarded as practically important. The smaller the difference you regard as important to detect, the greater thesample size required.

Factors such as time, cost, and how many subjects are actually available are constraints that often have to be taken in to account when designing a study, but these should not dictate the sample size - there is no point in carrying out a study that is too small, only to come up with results that are inconclusive and not generalizable to wider population.

There are two approaches to sample size calculations 1) precision based and 2) power based.

Precision based studies include, with what precision do you want to estimate the proportion, mean, prevalence or whatever it is you are measuring. (This article)

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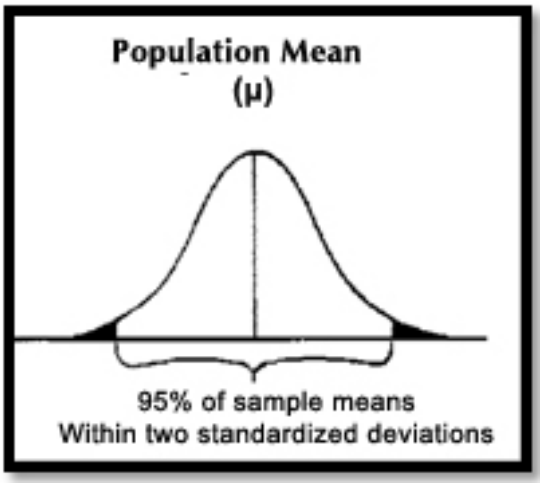

Fig.: Distribution of (sample) means of repeated samples from a population

Power based studies include, how small a difference is it important to detect and with what degree of certainty?

The level of precision: sometimes called as sampling error, is the range in which the true value of the population is estimated to be. This range is often expressed in percentage points (minimum of ± 5 to maximum of ± 10). Thus a researcher finds a 60 per cent sampled population have a disease 'x' with a selected precision rate of ± 5 percent, then the researcher can conclude that the disease prevalence would be between 55 and 65 in the population where he/she conducted the study.

The confidence level (risk level): Is based on the ideas which are contained under the central limit theorem. The key idea which is encompassed in central limit theorem is that when a population is repeatedly sampled, the average value of the attribute/the value of your interest by those samples is equal to the true population value. Furthermore, the values obtained by these samples are distributed normally about the true value, with some samples having higher value than the true population value (mean) and same number of samples having a lower value than the true population value. In a normal distribution, approximately 95 per cent of the sample values are within the two (2 SD cover is 95.45 per cent of the population value; 1.96 for an exact value of 95 per cent population value) standard deviation limits of the true population value (population mean).

In other words, this means that, if a 95 per cent confidence level is selected, 95 out of 100 samples would contain the true population value within the range precision specified (above figure). There is always a chance that the sample selected does not contain the true population value. Such samples with extreme values are represented by the shaded areas in the above figure. The shaded area amounts to 5 per cent (complement of 95 per cent) is known as level of significance which is denoted by α (alpha).

Example: The prevalence of anaemia among pregnant women is 60%. Let us assume the precision to be 5%. How many subjects are needed to be studied taking 95% confidence interval?

Proportion (p) of anaemia among pregnant women: 0.60

$\underline{\quad}$
dxd

(1 - p) = (1 - 0.60) = 0.40

Absolute precision: 5 per cent (0.05)

Confidence level (α)/interval: 95 per cent

α value (normal table value for a = 5%) : 1.96

Formula to calculate sample size:

$n = {}^{(Z(1-\alpha)2\,p\,(1-p)} = (1.962x\ 0.60\ x\ 0.40)/(0.05x0.05) = 369$

The final sample size is 369 to pick up the 60 per cent of anaemic pregnant women with a precision of 5 per cent. In other words the sample would be able to pick up anything between 55 to 65 per cent of prevalence of anaemia in pregnant females.

Here sample size was calculated for a single proportion study with a specified prevalence, level of precision. The precision can be altered to a higher or a lower level and accordingly we would get a lower or a higher sample size for the sample prevalence. It is totally depending upon the actual mean (population mean) and how much precision one would like to have. The final say about the sample size in this kind of scenario is that, the sample size depends upon the prevalence (population mean/earlier studies) and the level of precision (usually five percent or between two values, the population value would lie) one would like have.

There are other scenarios viz. single mean, two mean, two proportion, case-control, RCT, sensitivity, specificity, for which we will discuss in the next issue.

SUGGESTED READING

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GUIDELINES TO THE AUTHORS CONTRIBUTING TO SRI RAMACHANDRA JOURNAL OF MEDICINE

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- Lettering should in Times New Roman with a font size of 12.
- Three paper copies should be submitted to the editor.
- In addition to the paper copies, a digital copy should also be submitted through e-mail or on a compact disc.
- Studies involving human subjects or animals should have received the approval of the institutional ethics committee. A statement about this should be mentioned in the methods of the manuscript.
- Pictures having visible identity of patients should be accompanied by a duly signed patients consent form.

Authorship criteria :

Authorship credit should be based only on substantial contributions

1) to conception and design or acquisition of data or analysis and interpretation of data;
2) drafting the article or revising it critically for important intellectual content; and
3) final approval of the version to be published.

Conditions 1, 2 and 3 must all be met

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Articles of original research are welcome in this category. Articles should not exceed 4000 words. It must include an abstract of 250 words. Minimum of three MesH words to be mentioned at the bottom of the abstract. Upto 50 references may be included in these articles. The manuscript should be prepared as title page, abstract and keywords, introduction, materials and methods, statistical analysis, results, discussion, acknowledgement, references, tables and figures. Each of the above mentioned should begin in a fresh page.

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(ii) the initials followed by the name of each author and highest academic qualification;

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The authors are strictly advised not to mention their name and affiliation details in any of the subsequent pages other than the Title page since it may interfere with the review process.

II. ABSTRACT AND KEY WORDS: The second page should carry a structured abstract of not more than 250 words with subheadings of

(i) Background and objectives,

(ii) Methods,

(iii) Results and

(iv) Conclusions

It should be written for the readership of both clinicians and basic investigators and should state the hypothesis or central question of the study or investigation, the study subjects or experimental animals, observational and analytical methods, the main findings, and a final statement of the principal conclusions. Three to six key words using, where possible terms of medical subjects headings list from Index Medicus [MeSH].

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giving sufficient information to permit the work to be repeated. If a generally accepted technique has been used, only a reference to that is enough. If, however, such a technique has been modified by the workers, the manner in which this has been done should be clearly stated.

STATISTICAL METHODS:

The relevant statistical methods used for analysis should be briefly explained mentioning the objective of each statistical test in relation to the variables in the reported study that is meaningful. When 'p' value is mentioned the exact number should be mentioned [exception is a highly significant value which may be mentioned as <0.001]. Mention should be made about the predetermined level of 'p' value which will be considered significant. Details of the statistical software used and its version needs mention.

V. RESULTS. This section should not include materials suitable for inclusion in "Material and Methods' or "Discussion". The results should be presented in logical sequence in the text, tables and illustrations. The data presented in the tables or figures should not be repeated in the text. Only important and significant observations should be included.

VI. DISCUSSION. This should be limited to significance of results obtained and what can and what cannot be concluded and why. It should not be a repetition of the findings already given under 'Results'. Results should be discussed in the light of others' work in the field. Speculative and purely theoretical discussion to which results presented are not related will not be accepted.

VII. ACKNOWLEDGEMENTS: Acknowledgement should be brief and made specific for scientific/technical assistance and financial supports in the form of grants/drugs/ equipment only.

VIII. REFERENCES: References should be typed on a separate page after the text and these should be numbered consecutively in the order in which they are first mentioned in the text. In accordance with best practices in scientific writing, latest articles published in relevant area must be referenced. Identify references in text, tables, and legends by Arabic numerals in parentheses.

The titles of journals should be abbreviated according to the style used in Index Medicus. Consult the List of Journals Indexed in Index Medicus. The list can also be obtained through the library's web site (http://www.nlm.nih.gov).. List all the authors when there are six or fewer; but when there are seven or more, list the first six, then 'et al'. Examples of correct form of references are given here:

1. STANDARD JOURNAL ARTICLE

Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med 2002; 347: 284-7.

MORE THAN SIX AUTHORS:

Rose ME, Huerbin MB, Melick J, Marion DW, PalmerAM, Schiding JK, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res 2002; 935 (1-2): 40-6.

2. IN PRESS'

Tian D, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. Proc Natl Acad Sci USA. In press 2002.

BOOKS AND OTHER MONOGRAPHS

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Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. EuroGP 2002: 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale Ireland.

5. DISSERTATION

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002

6. ELECTRONIC MATERIAL:

Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2nd edition. Geneva : World Health Organization. available at: http://www.who.int/csr/resources/publications/dengue/Denguepublication/en/

[accessed on sep 10th 2008]

Correctness of the reference list is the entire responsibility of the author (s).

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FIGURES.:

(i) Glossy print photographs (in triplicate) are required (usually 10 cm × 8 cm); good contrast is essential for good reproduction.

(ii) Figures (not more than 4) may be submitted in a digital file, preferable in a JPEG (or) TIFF format.

(iii) Figures should be labeled appropriately using arrows [black, white, single or double] which should be mentioned and explained in the legend.

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(v) Legends should be provided for each figure, listed on a separate page.

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(i) Each table should be typed double-spaced on a separate sheet.

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(iii) They should have an underlined title followed by a legend, if any.

(iv) Explanatory matter should be in a footnote, not in the title. The symbols *,†,‡,§,||,¶,**,††,‡‡ can be used in the table or its foot note..

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Articles addressing an theme of current interest is welcome in this category. Articles should not exceed 4000 words. The manuscript should be prepared as title page, abstract and keywords,

introduction followed by discussion, acknowledgement, reference, tables and figures. Each of the above mentioned should begin in a fresh page

I. ABSTRACT AND KEYWORDS:

(i) In an unstructured format not more than 250 words.

(iii) It should describe the background and summary of the discussion related to the topic of interest.

(iii) Minimum of three MesH words to be mentioned at the bottom of the abstract. Upto 50 references may be included in these articles.

II. INTRODUCTION: It should commence on separate page and should briefly explain the reason for the review. This should be a brief overview about what is already known on the topic of the article. This should be followed by a statement on the method of review of literature. A systematic explanation of the methods followed to search the literature on the topic of interest is desirable.

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DESCRIPTION OF THE CASE AND DISCUSSION: The case should be narrated in a simple and logical manner with important observations shown as tables and figures [the latter two should be kept at the end of the manuscript as described earlier]. Discussion should focus on similar or related case reports published in the global literature and important or unusual features in the case described.

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Thoughtful discussions of current topics are welcome in this category. Should be no more than 500-1000 words, no tables or figures and references to a maximum of 10. The manuscript should be prepared as title page, abstract of 150 words with 3 Mesh terms, text of the manuscript which may be self styled followed by references

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Correspondence with comments on a recently published journal article or scientific content not related to a journal article [case report, research protocol, original research, etc] is welcome. Word limit is 300 words and 5 references. Abstract is not required. Title page should be as already described.

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