



Conference cum Workshop on 3RD ASIAN AND AFRICAN STEREOLOGY CONGRESS

On 18 -19 December, 2018

**Organised by
Department of Anatomy
Maulana Azad Medical College New Delhi**



Conference cum Workshop on
3RD ASIAN AND AFRICAN
STEREOLOGY CONGRESS

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**Maulana Azad Medical College &
Associated Hospitals:**
Govind Ballabh Pant Institute of Postgraduate
Medical Education and Research
Lok Nayak Hospital
Guru Nanak Eye Center

Dr. Sanjay Tyagi
M.D. (Medicine), DM (Cardiology)
FAMS, FICA, FESC, FACC (U.S.A.)
DEAN

Dated: 3rd December, 2018



DEAN'S MESSAGE

Maulana Azad Medical College takes pride in taking lead in training the professionals and sharing knowledge as well as experience.

I am extremely happy to know that the Department of Anatomy is conducting an International workshop on "3rd Asian and African Stereology Congress and Hands on Training Stereology Workshop, New Delhi" on 18th-20th December 2018.

I have been informed that the workshop has received an overwhelming response from various medical colleges of Delhi and other parts of the country, which indicates the interest in the field of stereology among the Anatomists.

I am sure all the participants as well as the organizers will be enriched by this learning exercise.

On this occasion, I wish the organizing committee and the participants all the best and hope they make the most of these two days of deliberations.

It gives me immense pleasure to extend my support for the workshop and wish it all the success.

(Dr. Sanjay Tyagi)

Prof. Suleyman Kaplan

President of Turkish Society for Stereology

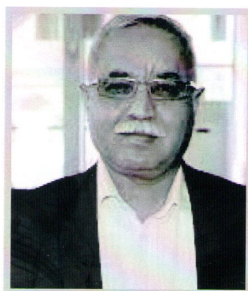
Board member of Journal Chemical Neuroanatomy (Elsevier)

Board member of Journal of Microscopy and Ultrastructure (Elsevier)

Head of Department of Histology and Embryology, Medical Faculty

Ondokuz Mayıs University

Samsun, Turkey



FOREWORD

This abstract book presents the abstracts of "3rd Asian and African Stereology Congress" focused on the subject of "stereological techniques" that take place in New Delhi, India from December 18th to December 20th, 2018. Maulana Azad Medical Collage and the Turkish Society for Stereology organized this congress. The main objective of the congress is to set up a discussion and sharing experiences platform on the practical application of stereological techniques. The first congress was held in Samsun, Turkey in the November 2012. The second congress, where many African, Asian, and European scientists participated as previously seen was held in Dubai, United Emirates in December 2015. The third congress of this series is organized in Maulana Azad Medical College in New Delhi.

The congress would provide information on the basic principles and practices of stereological techniques. The participants would benefit on the knowledge of current stereological software, interpretation of results on the cellular counting and volume estimation by stereological techniques, how can stereological techniques would be used in experimental and clinical studies. It is hoped that this congress would create a good environment for the scientists to communicate and cooperate with scientists that come from different parts of the world.

The program of this congress represents dedicated efforts of many people. We would like to express our gratitude to the mainly Prof. Sabita Mishra and members of the Organization Committee for their diligent and professional helps and supports. We thank to all of the speakers for their invaluable contribution and sharing their knowledge. We also thank to the Maulana Azad Medical Collage for the gentle hosts.

A successful congress that is new ideas of research projects are discussed and new collaborations are formed shows more than paper presentations in this congress. So I trust that we will present our best ideas to our colleagues and return home with a rich intellectual experience.

We wish you all of you an effective congress and an unforgettable memory in the historical and multicultural city of New Delhi. We hope to meet you again in next event that is 4th Asian and African Stereology Congress in a wonderful place in 2021.

Prof. Suleyman Kaplan

Presidents of Congress

New Delhi, India



Dr. Neelam Vasudeva
Director Professor
Department of Anatomy



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FOREWORD

Department of Anatomy, MAMC is committed in its endeavor for skill upgradation and knowledge sharing amongst health professionals. This International workshop, ***"3rd Asian and African Stereology Congress and Hands on Training Stereology Workshop, New Delhi"*** is another step in this series of knowledge sharing and interacting with our fraternity.

The workshop is organised in a manner to hone the skills of an Anatomist on Stereology techniques.

This three-day workshop is aimed at training faculty and residents through various scientific lecture sessions and hands on training activities under experts in the area of stereology.

I would like to thank Government of NCT of Delhi for their support, under the plan scheme "Strengthening of Medical Education & Training /Workshops at Maulana Azad Medical College by Govt. of NCT for conducting this workshop.

I am sure this will be a fulfilling experience for all of us.

Dr. Neelam Vasudeva
Director Professor
Organizing Chairperson
Organizing Committee



Dr. Sabita Mishra
Director Professor and Head
Department of Anatomy



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FOREWORD

It gives me immense pleasure to welcome you to the International workshop ***"3rd Asian and African Stereology Congress and Hands on Training Stereology Workshop, New Delhi"***

This workshop aims to provide hands on training to post graduate students and anatomists in basic and advanced stereology techniques and protocols.

The workshop would involve presentations by National & International experts, Preliminary hands on training and Poster presentation by participants. This will be followed by one day (20th Dec) hands on training on the Stereo Investigator at All India Institute of Medical Sciences, New Delhi.

I hope the workshop will be an enriching experience for both the participants and the organisers.

Dr. Sabita Mishra
Director Professor and Head
Organizing Secretary
Organizing Committee



शरीरमाद्यं खलु धर्मसाधनम्



ALL INDIA INSTITUTE OF MEDICAL SCIENCES NEW DELHI – 110 029

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Date: 30/11/2018

MESSAGE

I am pleased to be associated with the 3rd Asian and African Stereology Congress and Hands on Training Stereology Workshop to be held from 18-20th December 2018, in the Department of Anatomy, Maulana Azad Medical College (MAMC) New Delhi along with Department of Anatomy, AIIMS, New Delhi and the Turkish society of stereology. I believe there are a number of enthusiastic participants from all over India who are interested to learn advanced morphometric techniques. This will be a useful and reliable tool for all anatomists and neuroscientists.

I wish the organizers and participants to make this workshop a great success.

Dr. T S Roy

3RD ASIAN AND AFRICAN STEREOLOGY CONGRESS

18TH DECEMBER 2018, TUESDAY
(Maulana Azad Medical College, New Delhi)

08:00-08:45	Registration	
09:00-09:30	Inauguration: New LT Pathology Block	Anatomy Deptt
09:30-09:40	Tea Break	
	Session 1	
Chaired by:	Dr JM Kaul, Dr S Wadhwa, Dr S Kaplan	
Time	Topic	Speaker
09:40-10:05	Introduction to Stereology and sampling strategies	Suleyman Kaplan (Samsun, Turkey)
10:10-10:35	Applications of Stereology in Biomedical Sciences	Shashi Wadhwa (New Delhi, India)
10:40-11:05	Design based Stereology	Tony G Jacob (New Delhi, India)
11:05-11:15	Coffee Break	
	Session 2	
Chaired by:	Dr Smita Kakar, Dr Mahmut Ulubay, Dr P Sakhuja, Dr TC Nag	
11:25-11:40	Antigen localization in biological tissues: practical issue	T C Nag (New Delhi, India)
11:45-11:55	Substantia Nigra pars compacta of Asian Indians is partly protected against age-related degeneration	Phalguni Anand (Benguluru, India)
12:00-12:10	A stereological investigation of the effects of ellagic acid and silibin on dermal mast cells numerical density in rat skin exposed to ultraviolet A and B radiation	Seda Keskin (Van Turkey)
12:15-12:35	Effects of curcumin on the diabetic rat kidneys: A stereological study	Mahmut Ulubay (Samsun, Turkey)
12:40-13:00	Some empirical clues for physical and optical disector counting and preferred sectioning-staining methods in neuroscience	Kıymet K. Yurt (Samsun, Turkey)
13.00-14:00	Lunch	
	Session 3	
Chaired by:	Dr A Tuli, Dr S Raheja, Dr M Kohli, Dr Berin Z. Altunkaynak	
14:00-14:20	Using of the Cavalieri principle in clinical studies	Suleyman Kaplan (Samsun, Turkey)
14:20-14:35	Structural and ultrastructural changes of vasectomy on the bulbourethral gland: A rabbit experimental study	Awad, Khalid Amin (Khartum, Sudan)
14:35-14:45	Long-term exposure to 900 MHz electromagnetic fields induces oxidative stress but does not change axon number and myelination in developing rat sciatic nerve	Kıymet K. Yurt (Samsun, Turkey)
14:50-15:00	Folic acid protects rat kidney from the 900 MHz electromagnetic fields exposure	Omur G. Deniz (Samsun, Turkey)

15:00-15:15	Coffee Break	
	Session 4	
Chaired by:	<i>Dr S Aggarwal, Dr R Chauhan</i>	
15:15- 15:25	A stereological investigation of the curcumin effects on dermal vascular density in diethylnitrosamine-treated rats	<i>Murat Çetin Rağbetli (Van, Turkey)</i>
15:30-15:45	<u>Working of Neurolucida</u>	<i>Saroj Sharma (New Delhi, India)</i>
15:45-15:55	Tissue Processing Techniques for Stereology	<i>Dr Sabita Mishra</i>
15:55-16:15	Discussion on Practical aspects	<i>Sulyman Kaplan & Team</i>
16:15 onwards	Video-Conferencing with MBF Neurosciences	MBF Team, USA

19TH DECEMBER 2018, WEDNESDAY
(Maulana Azad Medical College, MAMC)

Time	Topic	Speakers
	Session 5	
Chaired by:	<i>Dr N Vasudeva, Dr Sohinder, Dr R K Saran</i>	
09:00-09:30	Stereological investigation of the developmental and age-related changes of the myelinated fibers in the human auditory nerve – A light and electron microscopic study	<i>T. S Roy (New Delhi, India)</i>
09:35-10:00	Three-dimensional Reconstruction of Neuron	<i>Soumya Iyengar (Manesar, India)</i>
10:05- 10:30	Effects of electromagnetic fields on the development of sciatic nerve	<i>Suleyman Kaplan (Samsun, Turkey)</i>
10:30-10:45	Coffee break	
	Session 6	
Chaired by:	<i>Dr G Rath, Dr T S Roy, Dr Pushpa Dhar, Dr Mustafa Kemal Atilla</i>	
10:50- 11:10	Cell Imaging and its quantification by morphometric tools	<i>Dr S B Ray (New Delhi, India)</i>
11:15-11:25	Possible neuroprotective effects of garcinia kola, thymoquinone and <i>Momordica charantia</i> on rat hippocampus exposed to 900 MHz electromagnetic fields for 2 hours a day during the prenatal period	<i>Omur G. Deniz (Samsun, Turkey)</i>
11:30-11:50	Potential effects of adipose tissue stem cells following sciatic nerve crush injury in rats: A stereological study	<i>Berin Z. Altunkaynak (Istanbul, Turkey)</i>
11:50-12:00	Coffee break	
Chaired by:	<i>Dr S B Ray, Dr Omur G. Deniz, Dr RK Suri, Dr Srijit Das</i>	
12:00-12:10	Investigation of the effect of curcumin and blue berry on axonal regeneration after peripheral nerve injury	<i>Arife Ahsen Kaplan (İstanbul, Turkey)</i>
12:15-12:25	The effects of some herbals on the rat hippocampus exposed to electromagnetic fields for one hour during prenatal period	<i>Omur G Deniz (Samsun, Turkey)</i>

12:30-12:40	Caffeine prevents neuronal loss in rats exposed to electromagnetic field	<i>Süleyman Kaplan (Samsun, Turkey)</i>
12:45-12:55	Measurement of the diameter of collagen fibre and wound area in experimentally induced diabetic Sprague Dawley rats	<i>Srijit Das (Malaysia)</i>
12:55-13:00	How To Improve Patient Satisfaction?	<i>Mustafa Kemal Atilla (Samsun, Turkey)</i>
13:00-14:00	Lunch	
	Session 7	
Chaired by:	<i>Dr Sabita Mishra, Dr I Alkan</i>	
14:00-14:15	Volumetric analysis of brain parts on magnetic resonance imaging	<i>Isinsu Alkan (Samsun, Turkey)</i>
14:20-14:30	Application of stereology in liver volumetric by CT scans	<i>Nidal Abubaker (Umdurman, Sudan)</i>
14:35-14:45	Application of stereology in neuroimaging of autism, epilepsy and schizophrenia	<i>Mohamed A/Salam Nurein (Umdurman, Sudan)</i>
14:50-15:00	Evaluation of the relationship between multiple sclerosis and obesity with quantitative magnetic resonance imaging: A stereological study	<i>Berin Z. Altunkaynak (Istanbul, Turkey)</i>
15:00-15:10	Coffee Break	
15:10-15:30	Presentation by Wolter Kluver	Wolter Kluver Team
15:30-16:00	Feedback & Valedictory of Conference	Anatomy Team
	High Tea & Cultural Programme	

HANDS ON TRAINING STEREOLOGY WORKSHOP

20TH DECEMBER 2018, THURSDAY TO BE HELD AT AIIMS
(All India Institute of Medical Science, New Delhi)

Reporting Times at AIIMS, New Delhi - 9:00a.m.		
9:30- 12:00	A practical method for the estimation of cell number: Physical and optical fractionator	<i>By AIIMS</i>
14:00-15:00	PRACTICE: Disector counting technique application	<i>By AIIMS</i>
15:15-16:15	PRACTICE: Fractionator application	<i>By AIIMS</i>
16:15-17:15	PRACTICE: Cavalieri application	<i>By AIIMS</i>
17:20-17:45	Feedback and closing ceremony	All

INTRODUCTION TO STEREOLOGY AND SAMPLING STRATEGIES

– Kaplan S

**DEPARTMENT OF HISTOLOGY AND EMBRYOLOGY, MEDICAL FACULTY, ONDOKUZ MAYIS
UNIVERSITY, SAMSUN, TURKEY**

Stereology is a science that can predict the morphological properties of three-dimensional objects by quantitatively analyzing their two-dimensional images. Thanks to the stereology, the information obtained about the structures is independent from observer biases and reliable. The evaluation of the data obtained from the light microscopy (conventional and confocal), electron microscopy and other types of microscopy, and also MRI, computerize tomography constitute a main basis for the stereological analysis. An estimation of length, surface area, volume, and number of objects for 3-D tissue structures in an organ would be provided by stereological techniques. With the systematic random sampling approach, researchers can make accurate and reliable predictions of morphological models with stereological analysis techniques. At the beginning of the study, the sampling process for a subject is designed by the researcher to the desired degree of precision. Sampling strategies are used in every step of the stereological estimation process, from the Cavalier's estimation of volume to the optical fractionator.

Key words: Stereology, Sampling, Disector, Fractionator, Cavalieri Principle, Unbiased

APPLICATIONS OF STEREOLOGY IN BIOMEDICAL SCIENCES

– Dr. Shashi Wadhwa

Ex-Professor and Head, AIIMS and NDMC, Delhi

Stereology involves applying a set of precise and simple tools to quantify properties such as volume, number, length and surface area of objects of interest in a three- dimensional structure from its 2D sections. The material is first sectioned and then a suitable test system of points, lines, sample frames etc. is randomly superimposed on the planar sections. The systematic random sampling of a structure with random placement of test-grid onto the images of interest allows an efficient and an unbiased quantitative analysis of biological tissue. This probing of a section is equivalent to direct probing of 3D space and is the mainstay of the stereological approach. Design-based stereology is regarded as the standard methodology for quantitative histology in biomedical research.

Stereology is the state-of-the-art research tool in neurosciences, toxicology, pathology and experimental situations. The 2D sections are obtained as microscopic slides (light microscope) or electron-micrographs of a solid specimen and X-rays or MRI's of the whole human body. It is used to measure subtle yet important alterations in structural morphology (gross, histological or ultrastructural) based upon disturbances during development, pathological changes in disease or experimental manipulations. It can also be applied to quantitate the ultrastructural or immunocytochemical changes in the cellular organelles in electron-micrographs. The structural changes so determined specifically identify the location of the functional alterations in the organ or tissue.

DESIGN BASED STEREOLOGY

– Tony G Jacob

Department of Anatomy, AIIMS, New Delhi

Stereology is a means of providing meaningful quantitative descriptions of the geometry of 3D structures from measurements that are made on 2D images (MJ West, 2011). It takes descriptive anatomy to the realm of scientific quantitation, in the same way as alchemy became chemistry through quantitative mathematics. When the element of bias is removed from quantitation it is then regarded as Design-Based Stereology (henceforth referred to as stereology). Stereology would become a prerequisite for the evaluation of biological tissues in comparative and experimental studies on tissues and organs. It uses elegant mathematical relationship equations that consider the loss of important structural features during the process of sectioning a tissue that is a pre-requisite in the evaluation of biological specimens. Estimates of a structural parameter with a statistically defined margin of error are adequate to achieve biologically relevant data. The margin of error can be decreased by the robustness of the sampling procedure.

When sectioning for design-based stereological estimates, it is mandatory that all parts of the structure of interest have equal probability of being present in the section that is eventually evaluated for analysis. This section should also be independent, for parameters like length and surfaces, of the orientation of the tissue as well. In three dimensions, any object in space is oriented with respect to the x, y and z axes. These are in layman's terms – length, breadth and height of the structure. A cube (volume = $l \times b \times h$, represented as m^3) has three dimensions, a rectangle has two dimensions (area = $l \times b$, represented as m^2), a line has only one dimension (length = l , represented as m - meter being the SI Unit) and a point has zero dimensions. In a section, solid structures become profiles, surfaces become lines, linear features become points and discrete objects become and unpredictable number of profiles. This indicates that structures lose one dimension of their orientation when sectioned. Hence, when probing for a parameter that has orientation in three dimensions, the sum of the dimensions of the structure of interest in the section and the probe should be equal to three, e.g. when counting objects in a three-dimensional structure, the probe would be volume based like a cube. Here the object being counted in profile has no dimension, and the volume probe has three dimensions, hence the sum of the dimensions of the parameters (number) and volume ($l \times b \times h$) is three. Similarly, when estimating the volume of a structure, the probe would be a point object that when scattered within the volume would each represent a unit volume that when counted within the boundaries of the structure would give an estimate of the total volume of the structure. Here, the structure has three dimensions (volume) and the point object representing unit volume has zero dimensions, hence the sum of the dimensions is again three.

Here, one would introduce the extremely useful dissector probe that enables us to count objects in a given volume. It essentially means two sections separated by a fixed distance that essentially encloses a volume. Now, modern stereology platforms, using objective lenses with high numerical aperture are able to probe into the depth of a thick section in order to count objects within the volume of the dissector probe that can then be extrapolated to the entire structure using systematic random sampling and back calculations. Here, the software takes care of the elegant but complex mathematical equations that not only give the estimates of the parameter but also the margins of error. Therefore, the researcher has to plan the approach to the estimation, exhaustively section the structure, randomly but systematically select the sections for evaluation and diligently apply the probes to the selected sections. The rest of the work is done by the software. It is hard and monotonous work, but the results obtained would be replicable and reliable given the experimental condition or even a disease state.

Design based stereology has been used extensively in neurobiological, pulmonary, renal, hepatic and stem cell research. Now, stereology platforms are also getting integrated with confocal microscopy systems that allow for optical sectioning of the tissue and are being integrated with anatomical mapping of brain areas and so on. This methodology may provide you the edge of reliability and reproducibility to the morphometric estimations in your research.

CELL IMAGING AND ITS QUANTIFICATION BY MORPHOMETRIC TOOLS

– S.B. Ray

Department of Anatomy, AIIMS, New Delhi

Cell imaging is a fundamental part of any teaching or research activity in Anatomy. Currently, apart from bright-field microscopy, which is the simplest form of imaging, fluorescence microscopy and the related confocal microscopy are being used extensively by research institutions in India and abroad. The difference between the two forms of microscopy is the setup of the microscope and the source of illumination – light in the visible range from a halogen or LED bulb versus high-energy emission bordering on the ultraviolet range from mercury or xenon burners. The latter technique uses a fluorescent dye for labeling specific components of the tissue. The maximum resolution of a bright field microscope is 0.2 microns. Fluorescence microscopy increases this resolution by enhancing contrast between the different cellular components. Technically superior to this is confocal microscopy, which also uses fluorescent dyes, but with a further increase in resolution and also comes with the provision of “Optical sectioning” of the tissue in the z-axis. There is the need to analyze and extract numerical information from the microscope images. In principle, the light microscope is well suited for measuring the size of well-resolved objects (Morphometry). This can be done at the basic level by taking images of a micrometer scale along with the images and calculating the factor of magnification. However, there are modern imaging software like the Nikon elements D, which automatically calculates the size of any object in the image using predefined measurement systems, integrated in the software. Densitometry or the determination of intensity of staining, can also be performed satisfactorily using these software packages or even by “Image J”, a freely downloadable software from NIH, USA. It can also be used for doing morphometric measurements. The part of the tissue to be assessed quantitatively can be manually selected using the tracing tool, a procedure known as segmentation. Cell, positively stained by immunohistochemistry, for the presence of a specific protein, can also be counted and then compared between control and the test (e.g. drug treated) groups. In fact, it can provide valuable data for supplementing images derived by immunohistochemistry.

SUBSTANTIA NIGRA PARS COMPACTA OF ASIAN INDIANS IS PARTLY PROTECTED AGAINST AGE-RELATED DEGENERATION

– Dr. Mrs. Phalguni Anand Alladi

*Department of Neurophysiology, National Institute of
Mental Health and Neurosciences, Bangalore, India*

Epidemiological studies on different ethnic populations worldwide report higher prevalence of PD in Caucasians compared to the Asian and African natives. Recent door-to-door studies conducted in India report relatively lower prevalence of Parkinson's disease (PD) in Asian-Indians. These findings may provide some evidence for ethnicity and cultural practices as important factors for the variable incidence of PD. Loss of melanized neurons of the substantia nigra pars compacta (SNpc) is an unfailing observation in PD. Aging being a major risk factor for PD and the finding of fewer melanized neurons in the nigra of PD patients led several investigators to quantify age-related loss of nigral neurons. In studies on aging French and American subjects, there was no age-related loss of nigral neurons, which was noted in the British Caucasians. In the American subjects, the number of melanized nigral neurons remained comparable during aging, though there was an exponential loss of tyrosine hydroxylase (TH) and Nurr1 co-labeled neurons. They proposed this to be an age-related loss of the dopaminergic phenotype, i.e. capacity to synthesize dopamine. In addition, there was an increase in α -synuclein expression from middle age.

To explore the possibility of ethnic differences in the vulnerability of dopaminergic SNpc neurons, we revisited the midbrains of Asian-Indians using principles of stereology and assessed the extent of melanization using cresyl violet stain. The expression of TH and Nurr1 was performed on immunohistochemically stained cryosections. Qualitative examination revealed gradual increase in neuromelanin in the SNpc from 1 year to adulthood. Furthermore, none of the age groups, showed evidence of apoptosis (Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling [TUNEL] positivity in dopaminergic SNpc neurons. Glial cell-derived neurotrophic growth factor (GDNF) receptors GFR α 1 and RET were seen to be expressed on the dopaminergic SNpc neurons through aging, suggesting GDNF responsiveness through life. The expression of ER stress markers and α -synuclein showed only a gradual increase. Both astrocytes and microglia showed morphological transformation, but no gliosis. Synaptic proteins were also preserved, so was the dendritic arborization. Thus distinct mechanisms may be responsible for SNpc neurodegeneration in aging and PD. The findings of relative stability in number and function of dopaminergic neurons may partly explain the lower prevalence of PD in the Asian Indian population compared with Caucasians.

A STEREOLOGICAL INVESTIGATION OF THE EFFECTS OF ELLAGIC ACID AND SILIBIN ON DERMAL MAST CELLS NUMERICAL DENSITY IN RAT SKIN EXPOSED TO ULTRAVIOLET A AND B RADIATION

– Keskin S¹, altındağ F¹, rağbetli MC¹

¹Department of Histology & Embryology, Faculty of Medicine Van Yuzuncu Yil University, 65080, Zeve Campus, Van, Turkey

Introduction : In this study, the protective effects of ultraviolet A and B radiation on dermal mast cells of Ellagic acid (EA) and Silibinin (SB) with antioxidant properties were investigated by stereological methods.

Materials & Methods : Six groups of 7 Wistar albino female rats were formed each. Groups; The control group (K), Ultraviolet AB group (UVAB), Ellagic acid (EA) group, Silibinin (SB) group, UVAB + EA (Ultraviolet AB + Ellagic acid) group and UVAB + SB (Ultraviolet AB+ Silibinin) group. The animals in UVAB, UVAB + EA and UVB + SB groups were exposed to UVAB radiation for 2 hours daily for 30 days. 50 mg/kg/day EA and 50 mg/kg/day SB were given orally 30 minutes prior to each UVAB application to the UVAB + EA and UVAB + SB groups. EA and SB without UVAB were given 50 mg/kg/day EA and SB by the same doses. All animals were sacrificed on the 30th day. Dermal mast cell numerical density was calculated by taking a sample of 12-15 samples from each rat ridge skin in 5 µm thickness. Kruskal-Wallis and Dunn's multiple comparison tests were used to compare the groups.

Results : Our results showed that the number density of mast cells decreased significantly compared to the UVAB group in the groups where EA and SB were combined with UVAB ($p < 0.05$).

Conclusion: UVAB radiation was observed to increase the number of dermal mast cells in skin tissue and EA and SB were effective in decreasing the number of dermal mast cells.

Key Words: Dermal mast cell, ellagic acid, numerical density, silibin, stereology, ultraviolet radiation.

(*) This study was taken from the Master Thesis Project (No: T7- 017-6175) and supported by Van YuzuncuYil University Scientific Research Projects.

EFFECTS OF CURCUMIN ON THE DIABETIC RAT KIDNEYS: A STEREOLOGICAL STUDY

– *Mahmut Ulubay¹, Işın Alkan², Kıymet Kübra Yurt², Süleyman Kaplan²*

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²Department of Histology and Embryology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

Introduction: The prevalence of the diabetes mellitus has been increasing in the world and the mortalities due to diabetes mellitus have become a common. The kidney disorders are among the most common disorders that are secondary to diabetes mellitus.

Aims & Objectives: The aim of study was to investigate the effect of curcumin on the kidney damage that was induced by the experimental diabetes mellitus model.

Material and methods: In the study, 56 female Wistar albino rats were divided into 7 groups: control group (Cont): no process was applied; Sham group (SH): corn oil was given via gavage for 14 days; Curcumin group (CURC): 30 mg/kg curcumin was given for 14 days as solved inside the corn oil; Diabetes group (DM): 50 mg/kg streptozotocin was given as one dose intraperitoneally for induction of diabetes; Diabetes+Curcumin group 1 (DC1): 7 days after the induction of diabetes 30 mg/kg curcumin was applied for 14 days; Diabetes+Curcumin group 2 (DC2): 14 days after the induction of diabetes 30 mg/kg curcumin was given for 14 days; Diabetes+Curcumin group 3 (DC3): 30 mg/kg curcumin application was performed simultaneously with diabetes induction it was continued for 14 days. At the end of experiment, the subjects were underwent intracardiac perfusion and the kidney tissues were excised. The harvested tissues were subjected to routine histological tissue processing and 5-micron thickness sections were taken systematic randomly. The sections were imaged and the medulla/cortex, tubule/glomeruli ratios were estimated.

Results: Qualitative observation of sections showed that general structures of kidney in diabetes mellitus groups were seriously impaired. A pronounced protection of curcumin, an antioxidant, was observed. Immunohistochemical and stereological analysis of kidneys have not been completed for all groups.

Discussion: Curcumin is an herbal component that is widely used in the traditional medicine in China and India. Curcumin, whose anti-inflammatory and anti-proliferative effects stand documented in several studies, has been reported to suppress the proinflammatory markers. It was also suggested that curcumin can do positively affecting the insulin resistance, hyperglycemia and hyperlipidemia in the diabetic patients. This study may confirm the positive effects of curcumin on the experimentally induced diabetic rat kidney.

Key words: curcumin, antioxidant, kidney, diabetes, stereology.

TISSUE PROCESSING TECHNIQUES FOR STEREOLOGY

– Dr. Sabita Mishra

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Most biological materials are made up of a large number of cells and tissues of varying size and shape. These are connected to form continuous three-dimensional organs. The common question that arises in each student or researcher is how a two dimensional image of a histology slide or an electron micrograph relates to a three dimensional structure to which it represents. To understand this concept a basic understanding of tissue processing for light and electron microscopy and the fundamental principles of light microscopy is essential.

To appreciate the microstructure of any tissue under a light microscope, the tissue has to go through a process of fixation, processing, sectioning and staining. Tissue processing aims to embed tissue in a solid medium firm enough to support and make it rigid to cut thin serial sections using a microtome; which can be subsequently stained and viewed under the microscope. Most commonly used embedding medium is paraffin wax for light microscopic studies. The process by which tissues are fixed, processed, embedded, and sampled have an impact on quantitative analysis. Method of tissue fixation also can affect the results. The type of fixative used can become important if additional analyses are desired (such as electron microscopy) or immunohistochemistry (IHC) to identify a certain cell type. Ideally 10% neutral-buffered formalin is used in most cases. Although paraffin is the ideal tissue-embedding medium, it can lead to underestimations of tissue volume due to a high degree of shrinkage. Frozen embedding is also done for some cases though it carries its own limitations.

The advent of modern semi-automated computer based stereology systems and the advances in microscopy have made stereology a practical laboratory method.

SOME EMPIRICAL CLUES FOR PHYSICAL AND OPTICAL DISECTOR COUNTING AND PREFERRED SECTIONING-STAINING METHODS IN NEUROSCIENCE

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A quantitative description of a three-dimensional (3D) object based on two-dimensional images can be made using stereological methods. These methods involve unbiased approaches and provide reliable results with quantitative data. The quantitative morphology of the nervous system has been thoroughly researched in this context. In particular, various novel methods such as design-based stereological approaches have been applied in neuroromorphological studies. The main foundations of these methods are systematic random sampling and a 3D approach to structures such as tissues and organs. One key point in these methods is that selected samples should represent the entire structure. Quantification of neurons, i.e. number of particles, is important for revealing degrees of neurodegeneration and regeneration in an organ or system. In order to obtain precise results by means of stereological analysis, counting items should be seen clearly in the tissue. If an item in the tissue cannot be seen, these cannot be analysed even using unbiased stereological techniques. Staining and sectioning processes therefore play a critical role in stereological analysis. The purpose of this review is to evaluate current neuroscience studies using optical and physical disector counting methods and to discuss their definitions and methodological characteristics. Although the efficiency of the optical disector method in light microscopic studies has been revealed in recent years, the physical disector method is more easily performed in electron microscopic studies. Also, it was offered some common basic staining and sectioning methods, which can be used for stereological techniques.

USING OF THE CAVALIERI PRINCIPLE IN CLINICAL STUDIES

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Stereological methods provide practical techniques for obtaining quantitative data about three-dimensional structures using two-dimensional images. These methods utilise various specific tools and sampling strategies to provide unbiased and efficient estimation in order to obtain a wide range of quantitative parameters including the number, size, volume, surface, length and density. Volumetric approaches of the stereological methods are very commonly used for clinical and research purposes. The Cavalieri principle is a simple, accurate, efficient and inexpensive stereological approach for volume estimation using histological sections or magnetic images of any organ or structures. Using this technique, the volume of any object could be estimated from a set of slices through the object, provided that they are parallel, separated by a known distance. The cut surface areas of the sections are assessed and multiplication of the total cut surface area with the mean of the section thickness provides an estimation of the volume of the examined object. The volumetric change is an indicator of an organ's health status. The Cavalieri principle estimates the total volume of an organ and its subcomponents more accurately and precisely than the other methods. It runs simply and easily and produces efficient results.

There are numerous studies describing Cavalieri principles. However most of them contain very intricate information about the technique. In the present presentation, we aimed to give clear and detailed information on the applications of the Cavalieri principle and discuss the importance of the volume estimation in biomedical sciences.

Key words: Brain, Cavalieri principle, Quantitative, Stereology, Unbiased

STRUCTURAL AND ULTRASTRUCTURAL CHANGES OF VASECTOMY ON THE BULBOURETHRAL GLAND: A RABBIT EXPERIMENTAL STUDY

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Introduction : Vasectomy has been defined as removal of a segment of the vas deferens for the purpose of male sterilization. The aim of this study is to outlines the morphological changes in male rabbit bulbourethral gland associated with vasectomy under light and electron microscopes.

Material and methods : Eleven male New Zealand rabbits were used in this study; eight for vasectomy and three as normal control. Bilateral vasectomy was carried out after ligation with 4-0 non-absorbable suture materials. Animals were divided into four groups (two animals in each group). The bulbourethral gland is sampled after 24, 28, 32 and 36 weeks after vasectomy according to the respective groups. Small pieces of tissues were used for light and transmission electron microscopy.

Results : The control animals showed crowded glandular parenchyma embedded in bundles of striated muscle. With vasectomy the changes noted are mainly in the epithelium that became increasingly cuboidal. With 36 weeks of vasectomy the epithelium became low cuboidal. Vasectomy decreased the amount of collagenous fibers especially after 30 weeks onward and resulted in changes in the shape of the acinar cells and reduction in the number of electron dense granules.

Conclusion : Vasectomy has structural and ultrastructural changes on the bulbourethral gland. These changes include lowering the height of the lining epithelial cells and decreasing in the apical secretory granules. These changes may indicate decreased activity of the bulbourethral gland with vasectomy.

Key words: Bulbourethral gland, vasectomy, rabbit, histology and ultrastructure.

LONG-TERM EXPOSURE TO 900 MHZ ELECTROMAGNETIC FIELDS INDUCES OXIDATIVE STRESS BUT DOES NOT CHANGE AXON NUMBER AND MYELINATION IN DEVELOPING RAT SCIATIC NERVE

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Introduction : Exposure to prolonged EMF during the prenatal period may lead to irreversible damage to the nerve tissue. It may also have an adverse effect on the oxidant / antioxidant status. In this study, the effects of long-term EMF exposure in the prenatal period of the rat sciatic nerves were evaluated quantitatively, biochemically and histopathologically. At the same time, the effects of some antioxidants such as Garcinia cola, Momordica charantia and Thymoquinone were investigated.

Material & Method : Twenty-seven pregnant Wistar albino rats were divided into 9 groups. The EMF groups was exposed to 900 Megahertz (MHz) EMF for 1 hour per day, for 21 days. Control, Sham, Garcinia cola, Momordica charantia and Thymoquinone groups were not subjected to EMF. Samples taken from right sciatic nerve tissues 4 weeks after birth were evaluated by stereological and histopathological methods. Mean numbers of myelinated axons, axon cross-section areas and the mean thickness of the myelin sheet were estimated with stereological methods. In addition, activities of enzymes such as catalase (CAT) and superoxide dismutase (SOD) were determined in blood samples.

Results : There were no differences between the groups regarding the mean number of myelinated axons, axon cross-section areas and mean thickness of the myelin sheet ($p>0.05$). CAT and SOD enzymes activity were significantly increase in the 900 MHz EMF exposed group ($p<0.05$). Also, according the TEM results Garcinia cola and Thymoquinone has shown toxic effect in EMF exposed groups.

Conclusion : According the results we suggest that EMF exposure can disturbs the balance between antioxidants and reactive species, making some antioxidants harmful.

Keywords : Prenatal, Stereology, Garcinia cola, Momordica charantia, Thymoquinone, Antioxidant, EMF.

FOLIC ACID PROTECTS RAT KIDNEY FROM THE 900 MHZ ELECTROMAGNETIC FIELDS EXPOSURE

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Introduction: Due to the widespread use of mobile phones, exposure to electromagnetic radiation (EMR) in everyday life has increased. The main aim of study was to investigate the protective effects of folic acid against adverse effects of 900 MHz EMF exposures on rat kidney.

Material & Method : 24 adult male Wistar albino rats were divided into three groups as Cont group, EMR group, EMR+FA group and FA group. In addition, each group consists of six rats. EMR group and EMR+FA group were exposed to EMR for 1 h/day during 21 days. Following the experimental and routine histological procedures, the Cavalieri principle was used for the estimation of mean volume of kidney, medulla and cortex, proximal and distal tubules. Also, the physical disector was used for estimation of the glomeruli number. IBM SPSS Statistics V21.0 were used for statistical analyses.

Result: A significant increase in the mean volume of the cortex, medulla, proximal and distal tubules of EMR group compared to Cont group. The total number of glomeruli in the EMR group was decreased compared to the Cont group. A significant increase in the total number of glomeruli EMR+FA group was found compared to EMR group. The mean volume of cortex and medulla in the EMR+FA group was significantly lower than EMR group.

Conclusion: The exposure to EMR causes to tissue damage in the rat kidney. In addition, FA treatment can cause to protective effects on the cortex and medulla volume and glomeruli number of kidney exposed to 900 MHz EMF.

Keywords: Electromagnetic field, folic acid, physical disector, Cavalieri method, renal damage.

A STEREOLOGICAL INVESTIGATION OF THE CURCUMIN EFFECTS ON DERMAL VASCULAR DENSITY IN DIETHYLNITROSAMINE-TREATED RATS

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Introduction : Effects of diethylnitrosamine (DEN) and curcumin on the dermal vessels length intensity dimension was investigated.

Materials and Methods : Adult Wistar albino rats were used from the experimental animal group of Van YüzüncüYıl University. Four groups were allocated as six in each group. The first group was administered with saline 0.5 ml/kg/ip on the first day and 0.5 ml/kg with gavage for 15 days (GI). The second group, curcumin, was given at a dose of 200 mg/kg/day for 15 days with 0.5 ml/kg gavage (G II). On Day 5, a single dose of 150 mg/kg/ip DEN was injected in (G III). The fourth group was given a single dose of 150 mg/kg/day for 5 days and curcumin 200 mg/kg/day for 15 days (G IV). At the end of the experiment, the animals were perfused, the skin pieces near the back of the head were removed and the tissues were tested. For routine follow-up to determine capillary length density. Every 30th section was taken up to the end of the tissue in 5mμ thickness. The mean obtained ten sections were examined stereologically evaluation of length density data from dermal images.

Results : Our results showed that no significant differences between the groups that were compared with the Man Whitney-U test regarding the length density groups.

Conclusions : In the study, the effect of curcumin on the length of the dermal veins was discussed in the literature.

Key words: Skin, Stereology, Unbiased, Curcumin, Diethylnitrosamine.

WORKING OF NEUROLUCIDA

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Neurolucida is a scientific system for neuron tracing and reconstruction, neuron analysis and 3D brain mapping. It provides detailed morphometric analysis of neurons by quantifying the whole dendritic arborisation which includes number, length, width and volume of dendrites and shape, number and length of spine and helps in elaborating the complexity of neuron network.

Neurolucida supports live digital video and acquired images from multiple image modalities, such as brightfield, fluorescence, confocal, electron microscope and scanning tomographic sources. It controls a motorized XYZ stage for integrated navigation through tissue sections and records each data point in 3D space. It acquires data through multiple Z levels, capturing the full 3D extent of neurons and brain regions.

One of the methods by which neuronal processes may be demonstrated is by staining the tissue sections by the method first described by Golgi, which works on the principle of metallic impregnation of cell cytoplasm.

The data is collected using Neurolucida Explorer which is a companion program of Neurolucida. It provides an automatic analysis of hundreds of quantitative parameters which helps in deconstructing complicated 3D anatomical features. This is extensively used in the field of Neurosciences including neuropathy, memory, behaviour studies, aging and in various neurodegenerative diseases.

STEREOLOGICAL INVESTIGATION OF THE DEVELOPMENTAL AND AGE-RELATED CHANGES OF THE MYELINATED FIBERS IN THE HUMAN AUDITORY NERVE – A LIGHT AND ELECTRON MICROSCOPIC STUDY

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The decline of hearing during normal aging is associated with neuronal death and synapse loss in the spiral ganglion. Until now, age-related changes in the myelinated fibers of the human auditory nerve have not been investigated. Therefore, the myelinated fibers in the auditory nerve of fetus, young (0-30 yrs), middle-aged (31-50 yrs) and old (above 51 yrs) human were studied with stereological methods and transmission electron microscopy.

The results showed that myelination of auditory nerve initiates around 20th week of gestation and total number of fibers reaches up to 40000 at birth. Aging affects the total number, area and mean diameter, myelin thickness of the nerve fibers in the auditory nerve.

Our results suggest that the age-related decline in the total number of nerve fibers, fiber thickness and other changes in the nerve fibers may contribute to functional changes in the auditory system leading to presbycusis.

THREE-DIMENSIONAL RECONSTRUCTION OF NEURONS

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Three-dimensional reconstruction of neurons and axons remains a powerful tool to study the details of their morphology in different parts of the brain. Provided that the tissue used for reconstruction is treated uniformly during fixation, sectioning and staining, it is possible to study neuronal microstructure across development and between normal/control and disease processes. While manual reconstruction is still performed, it is laborious and time-consuming. A number of methods have been developed to digitizing and automating the process to collect data on various parameter of dendrites and axons such as their length, branch number and thickness, number of varicosities and spine density. Further, the volume of dendritic trees, neuronal somata and axonal terminal fields can also be reconstructed. Combined with the recent advances in cellular imaging and microscopy, automated reconstruction of the morphology of neurons is very important for furthering our knowledge of the myriad connections of the brain in different states and across different organisms.

EFFECTS OF ELECTROMAGNETIC FIELDS ON THE DEVELOPMENT OF SCIATIC NERVE

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Introduction : An evidence of the negative impact of electromagnetic field (EMF) exposure on human health is on the rise. Unfortunately, studies that report prenatal damage caused by EMF exposure have also become widespread. In this study, effects of EMF exposure to the developing sciatic nerve and the potential role of some antioxidants were investigated by stereological and biochemical methods.

Material & Method : Twenty-seven pregnant Wistar albino rats were divided into 9 groups. EMF groups was exposed to 900 Megahertz (MHz) EMF for 2 hours per day, for 21 days. Control, Sham, pure *Garcinia cola*, *Momordicacharantia* and Thymoquinone groups were not subjected to EMF. Samples taken from right sciatic nerve of 4 week-old after birth were evaluated. Activities of catalase (CAT) and superoxide dismutase (SOD) were determined in blood samples.

Results : There were no differences between the all groups regarding the mean number of myelinated axons ($p>0.05$). In the EMF+*Garcinia cola* group, axon cross-section areas and mean thickness of the myelin sheet was significantly reduced than EMF and Cont group, respectively ($p<0.05$). CAT and SOD enzymes activity were significantly increase in the EMF exposed group while it was found a reduced enzymes activity in the EMF exposed antioxidant groups ($p<0.05$).

Conclusion : According to these results, prenatal EMF exposure can have indirectly harmful effect on developing sciatic nerve. It is recommend avoiding EMF exposure during the prenatal period as much as possible, and should be careful with the use of some exogenous antioxidants.

Keywords : Prenatal, Nerve fiber, Rat, Stereology, *Garcinia cola*, *Momordicacharantia*, Thymoquinone.

ANTIGEN LOCALIZATION IN BIOLOGICAL TISSUES: PRACTICAL ISSUES

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Antigen localization in biological tissues is a routine laboratory/research method by which we determine the presence of specific antigens (proteins, hormones, receptors, neurotransmitters and growth factors etc.) in a tissue. Their presence, thus, determines the functional activity of the cells that characterize the tissue. It is, therefore, of critical importance to be assured that the results of antigen localization are true and not "false-positive". The problem of false-positivity is common and faced by many workers even by adopting the standard methods of immunohistochemistry. Assuming that the post-mortem antigen preservation in a tissue is satisfactory, the problem of false-positivity may stem from variations at several steps in immunolabeling these may be right in the initial error of correct selection and nature of antibodies used for the detection of antigens. Antibodies that are well-characterized by previous workers need to be chosen in antigen localization. In cases where the available antibodies are not characterized properly, this can simply be performed by adsorption of the antibody with a control peptide/substance against which it was generated. The adsorbed antiserum-control is then applied to tissue sections. No reactions are expected, provided that the antibody used is pure and target specific. The second hurdle in achieving true immunolabeling often centers on the use of right dilutions of the antibodies and it is the responsibility of the workers to find out in a rigorous way the correct antibody dilution that gives an optimal, true labeling of the target and not unwanted, false-positive labeling in additional cells. In immunoperoxidase reaction, it is critical that the reaction is carried out for a considerable period with lowest concentrations of substrate and chromogen, so that intensity of reaction can be understood and checked by observing the slide under a microscope. Non-specific, false-positive reactions often result due to use of concentrated chromogen (e. g, DAB) and substrate (hydrogen peroxide). In immunofluorescence labeling, the use of a diluted secondary antibody conjugated to a fluorochrome(e.g., FITC) can give specific signals, and once determined, better labeling can be obtained by further dilution of the antibody and repeated washing. The immunofluorescence labeling may show autofluorescence, and this can be minimized by treatment of the sections in copper sulfide solution after incubation in primary antibody.

POSSIBLE NEUROPROTECTIVE EFFECTS OF GARCINIA KOLA, THYMOQUINONE AND MOMORDICA CHARANTIA ON RAT HIPPOCAMPUS EXPOSED TO 900 MHZ ELECTROMAGNETIC FIELDS FOR 2 HOURS A DAY DURING THE PRENATAL PERIOD

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Introduction: The effects of EMF exposure on the central and peripheral nervous system are very important issue because of the close usage of mobile phones to the brain and other organs. The main aim of this experiment was to investigate the effects of antioxidant effects of garcinia kola (GK), thymoquinone (TIM) and momordica charantia against negative effects of the EMF exposure.

Material & Method: Twenty seven Wistar albinoof pregnant rats were divided into 9 equal groups: Cont group, Sham group, EMF group, EMF+TIM group, EMF+MC group, EMF+GK group, TIM group, MC group and GK group. EMF groups were exposed to 900 MHz EMA for 2 hours daily for during the gestation period of (21 days) rats. After birth, at the postnatal 28-day, behavioral tests were made to analyze the learning and memory functions. After routine histological procedures, optical fractionator method was used for neuron counting. Biochemical tests were performed for determining the oxidative stress.

Result : A significant decrease in the neuron number of the EMF group was found compared to the Cont group. Additionally, a significant cell protection was observed EMF+TIM group, EMF+MC group, and EMF + GK group in comparison of to EMF group.

Conclusion: Exposure to 900 MHz EMF during the prenatal period may cause adverse changes in the brain. In addition, GK, MC, and TIM can reduce these adverse effects. The mechanisms underlying the adverse effects of TIM and GK on the hippocampus when are applied alone should be investigated.

Keywords: Electromagnetic fields, optical fractionator, oxidative stress, neuronal loss, memory deficit.

POTENTIAL EFFECTS OF ADIPOSE TISSUE STEM CELLS FOLLOWING SCIATIC NERVE CRUSH INJURY IN RATS: A STEREOLOGICAL STUDY

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Introduction: Cell-based therapy is a strategy to create a favourable environment for nerve regeneration in both the central and peripheral nervous system. Peripheral nerve damages result in poor sensory recovery. Surgical methods are alone not sufficient to overcome these sensory losses. These methods alone cannot cope with cell deaths. Therefore, stem cell-based treatment approach has been considered that it may overcome losses and support axonal regeneration positively.

Materials and Methods: In this study, thirty Wistar Albino female rats were separated into 6 equal groups; non-obese control (NOC), obese control (OK), non-obese injury (NOH), obese injury (OH), non-obese adipose (NOY), obese adipose (OY). At the end of 8 weeks, all experimental animals were subjected to nerve crush procedure, then fat stem cell homogenate was injected and then recovery process has been observed. After regeneration process, sciatic nerves of all groups were dissected, and histopathological, stereological and electrophysiological analyses were made.

Results: Stereological results showed that fat homogenate gave successful result in injury group in terms of myelinated axon number. The number of unmyelinated axons was increased with fat homogenate in both of the injured groups. When obese injury group and group with fat homogenate were compared, it has been determined that myelin sheath thickness increased with treatment ($p < 0.01$). From ENMG results, in terms of latency values, in the groups damaged but treated with fat-based stem cell, it decreased significantly; in other words, the message was delivered at a shorter time.

Conclusion: The all results showed that stem cell treatment could be successful on the peripheral nerve regeneration.

Keywords: Sciatic nerve, Mesenchymal Stem Cell, Obesity, Rat, Nerve regeneration.

INVESTIGATION OF THE EFFECT OF CURCUMIN AND BLUEBERRY ON AXONAL REGENERATION AFTER PERIPHERAL NERVE INJURY

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Introduction: Peripheral nerve injuries occur commonly loss of motor, sensory and autonomic functions of nerves as a result of traumatic conditions. The injuries have negative consequences and the frequent occurrence indicates the necessity of studies to minimize and cure nerve damage. This study aimed to analyse the axonal regeneration and healing effects of curcumin and blueberry using stereological and electrophysiological methods, after peripheral nerve injury.

Material and Methods: After sciatic nerve crush injury, rats were treated with ip injection of 30 mg/kg curcumin (Sigma C1386) and with gavage (4 g/kg blueberry) (Üçel Helvacılık) during 4 weeks. 14th and 28th day after injury SFI analysis were carried out and EMG was recorded. Under light microscope stereological analysis and electron microscopy observation were performed.

Results: There were not any significant differences between crush group and curcumin, blueberry. There are no significant differences between groups in SFI analysis. In the electromyography (EMG) test, there was a difference between the blueberry group and the crush group, $p < 0.05$, but no significant difference was found between the crush group and curcumin group. In electron microscopic analysis, protective effect of curcumin and blueberry treatment after injury was observed.

Conclusion: Significant effect of blueberry and curcumin on axonal regeneration could not be determined. Due to the low bioavailability of curcumin and the low dosage of blueberry, the significant effects on axonal regeneration may not be determined.

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THE EFFECTS OF SOME HERBALS ON THE RAT HIPPOCAMPUS EXPOSED TO ELECTROMAGNETIC FIELDS FOR ONE HOUR DURING PRENATAL PERIOD

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Introduction: It was aimed to highlight the possible effects of the electromagnetic fields (EMF) emitted by the mobile phones on the hippocampus and to investigate whether these possible effects can be reduced with various antioxidant substances.

Material & Methods: Forty-two adult Wistar albino female rats were divided into 14 equal groups with 3 pregnant in each group. The study groups of EMF were exposed to 900 Megahertz (MHz) EMF for 21 days, 1 hour (hr) per day. Also, the rats in the groups of Control (Cont), *Garcinia kola* (GK), *Momordicacharantia* (MC) and thymoquinone (TQ) were not exposed to EMF. While the Sham groups were kept in the EMF exposure system, they were not exposed to EMF. Four weeks after birth, rats were subjected to behavioural tests. After that, the samples taken from the brain tissues of the rats were evaluated with histological, stereological and immunohistochemical methods. The numbers of pyramidal neurons of hippocampus in rat cornuammonis (CA) were estimated with optical fractionator method. Moreover, the activities of the superoxide dismutase (SOD) and catalase (CAT) enzymes were evaluated biochemically in the blood samples.

Result: The analysis indicated that the total pyramidal neuron number in the CA of EMF (1 hr) group decreased significantly ($p < 0.01$). Also, the results showed that the protective effect of MC was more potent than the other antioxidant substances ($p < 0.01$).

Conclusion: 900 MHz EMF can cause hazardous changes in the brain. At this point, it can also be suggested that GK, MC and TQ can reduce these hazardous effects.

Key words: Stereology, Optical Fractionator, *Garcinia kola*, *Momordicacharantia* and thymoquinone, Brain.

CAFFEINE PREVENTS NEURONAL LOSS IN RATS EXPOSED TO ELECTROMAGNETIC FIELD

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Introduction: Electromagnetic field (EMF) emitted by cell phones has been shown to causes a decrease in the neuron number in many parts of brain. In this study, the protective effect of caffeine on the neuron loss in the animals exposed to EMF was investigated.

Material & Method: This study included in 5 groups each with 6 animals. The first group was exposed to 900 MHz EMF for 60 minutes per day, for 28 days and fed with water containing 1 mg/L caffeine. The second group was exposed to EMF but fed with tap water. The third group was not exposed to EMF and fed with caffeine containing water. The fourth group served as sham and kept in the EMF device without applying EMF. The fifth group was control and was not subjected to any procedure. After 28 days, the brain and cerebellar tissues of the rats were removed for histological processes. Effects of caffeine on neuron numbers in hippocampus and brain were investigated with stereological methods.

Results: The results show that there was a significant decrease in the number of Purkinje neurons of the cerebellum and pyramidal neurons of the hippocampus in rats exposed to EMF. In rats received caffeine, exposure to EMF did not cause a significant decrease in neuron number in hippocampus and cerebellum.

Conclusion: The results suggest that caffeine can reduce neuronal loss in the cerebellum and hippocampus in ratsexposed to electromagnetic field.

This study was supported by TÜBİTAK with a project number 214S642.

Key words: Caffeine, Electromagnetic field, Cerebellum, Stereology, Neuron number, Cell phone.

MEASUREMENT OF THE DIAMETER OF COLLAGEN FIBRE AND WOUND AREA IN EXPERIMENTALLY INDUCED DIABETIC SPRAGUE DAWLEY RATS

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Diabetes mellitus results in various complications which are difficult to treat. Delayed wound healing is one of the main challenges. Over the past decade, we created experimental animal model of diabetes and treated them with various natural products. Wounds were serially photographed using a digital camera (Coolpix 5400; Nikon, Tokyo, Japan) on days 1, 5, and 10 after wounding. The wound area was measured using image analysis software (VideoTestMaster Morphology; VideoTest, St Petersburg, Russia). A formula was used to calculate the rate of wound closure. A faster rate of wound closure showed the evidence of protective action of any natural product. The wound tissues collected from the experimental animal were processed for electron microscopy. Electromicrograph of the granulation tissue of the wound was taken with a transmission electron microscope Tecnai G2. Using the same software, the diameter of the collagen fibres was calculated. A decrease in the diameter of the collagen fibre was seen in diabetic wounds compared to the normal wounds. The method for measurement of the diameter of collagen fibre is being discussed in detail.

Keywords: Diabetes mellitus, wound, measurement.

HOW TO IMPROVE PATIENT SATISFACTION?

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Aim: To determine the factors affecting patients' attitudes towards health care satisfaction.

Materials and Methods: A detailed searching for the literature and studies was made.

Results: The most prominent factors affecting patients' satisfaction were found as communication between caregivers and patients, informing patients about the procedures, kindness and cleanliness of staff.

Conclusion: Health care is an integrated not only a medical but also a social system affecting on both sides-caregivers and recipients. Basic graciousness and kindness along with a correct communication with patient will provide satisfaction.

VOLUMETRIC ANALYSIS OF BRAIN PARTS ON MAGNETIC RESONANCE IMAGING

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Morphological, comparative and experimental studies are focusing on obtaining a reliable and accurate data from the concerning tissue. An estimation of volume belongs to a group of subject is a crucial criteria for biomedical sciences. It raises the reliability of the collected data. The alteration in the volume gives some clues of the organ's health. Especially in the investigation of the brain functions, the volume and surface area of it are counted as important parameters. At this point, quantitative data can be obtained by utilizing the efficient and unbiased methods provided by the stereological techniques. Recently, there is an increasing in number of the studies focusing on the brain. Especially in the diagnosis affecting the central nervous system, it is a crucial to know the volume and the anatomic features of the affected structure. For this reason, imaging techniques such as computed tomography; magnetic resonance imaging are frequently used when the stereological methods are applied.

Quantitative measurements provide more definite diagnoses in the medical field. These can be stored, transported, and analyzed easily than the qualitative ones. Stereological techniques are most effective methods for volume estimation.

Key words: Cavalieri principle, Stereology, MRI, Computed tomography, Brain, Morphology.

APPLICATION OF STEREOLOGY IN LIVER VOLUMETRIC BY CT SCANS

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Introduction: The need for stereology in liver conditions is becoming essential. It is required for liver volume determination; follow up of liver mass and assessment of gall bladder volume. Since the liver shape is not regular so stereology is the exit of this dilemma. Different stereological techniques were used in measuring the volume of the liver. Of these is the planimetry procedure, which concerned with demarcation of the liver boundaries and measuring it by the aid of diverse soft wares. Computerized topographic scan with contrast is considered as a reliable method in liver volumetry when compared to magnetic resonance images, which may give a poor demarcation for liver boundaries. This study aimed to use stereology in measuring the volume of the liver by designing a protocol then applying it in the measurements.

Methodology: Total liver volume of 300 individuals was measured using CT scan abdomen with contrast obtained for those without any hepatobiliary disease. A protocol was designed and validated for the measurements. Three different software were used in the process of measurement included MRIcrow, Imagej and Onis 2.6.

Results: The mean liver volume in Sudanese population was estimated by about $3261.32 \text{ cm}^3 \pm 1365.313 \text{ SD}$. There was a highly significant correlation between liver volume and body mass index ($P \text{ value} = 0.001$) and also with weight ($P \text{ value} = 0.000$) in both genders. However, no statistical significance between liver volume and height.

Conclusions: Liver volume varies with age, body mass index and may be racial or environmental.

APPLICATION OF STEREOLOGY IN NEUROIMAGING OF AUTISM, EPILEPSY AND SCHIZOPHRENIA

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Introduction: Stereology is the science of obtaining three-dimensional data from two-dimensional images. The data include volume, size, and length. It is used in objects of irregular shape where the ordinary mathematical rules could not be applied. The brain is a typical example of these structures. Conventional brain imaging failed to display abnormalities in autism, epilepsy, and schizophrenia. Advances in neuroimaging produce 3D brain scans through MRI and CT, which are used in stereology. This study explored changes in volume and size of brain structures related to autism, epilepsy and schizophrenia. It included results of an original research of MRI volumetry in temporal lobe epilepsy and a review in neuroimaging of autism and schizophrenia.

Methodology: The procedure of volume measurement in temporal lobe epilepsy is described with a focus on image acquisition. MPRAGE in 1.5T scanner, one mm slice-thickness were used. ROIs were delineated using planimetry. Findings in all the conditions were compared to matching control groups.

Results: In epilepsy, loss of lateralization in temporal lobe, superior temporal gyrus and volume reduction in hippocampus and entorhinal cortex. In autism, a reduction in temporal lobe volume amygdala, hippocampus, cerebellum, and inconsistent findings in the parietal lobe were observed. In schizophrenia alterations in size of corpus callosum with a reduced volume of the cerebral cortex, temporal lobe, hippocampus, D LPFC cerebellum, auditory gyri dilatation in lateral ventricles and enlargement of white matter were seen.

Conclusion: Stereology has given a widened understanding in the pathophysiology of neurological and psychiatric conditions based on quantitative data.

EVALUATION OF THE RELATIONSHIP BETWEEN MULTIPLESCLEROSIS AND OBESITY WITH QUANTITATIVE MAGNETIC RESONANCE IMAGING: A STEREOLOGICAL STUDY

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Introduction: In young adults, multiple sclerosis (MS) is an autoimmune inflammatory disease that occurs influenced by genetic and environmental factors. Axonal loss occurs in the chronic phase of MS. Recent studies focused on the relationship between MS and obesity. The main aim of our study was to observe the effects of obesity on MS using objective stereological methods in brain magnetic resonance (MR) imaging in obese MS patients.

Materials and Methods: Our study consisted of two groups; non-obese MS patients (n = 28) and obese MS patients (n = 27). Individuals with a body mass index (BMI) between 18.5-24.9 were considered overweight, and a BMI of 30.0-39.9 were considered obese. In our study, data were collected by scanning the files of MS patients from the Neurology Department of OndokuzMayis University Medical School. According to BMI, brain MRIs were examined in obese and non-obese MS patients and sclerotic plaque, gray matter and white matter volumes were estimated using the Cavalieri principle. For statistical evaluation, SPSS program (version 21.0; SPSS Inc., Chicago, IL, USA) was used. In the intergroup evaluations, p < 0.05 difference was considered significant.

Results: Stereological analysis revealed that there was no significant difference in the gray and white matter, total brain volume between non-obese and obese MS patients, however, the plaque volume in the obese group increased significantly (p < 0.01).

Conclusion: Obesity plays a role in the pathogenesis of MS. Prevention of obesity, especially during childhood and adolescence, can significantly reduce the risk of illness.

Keywords: Magnetic Resonance Imaging; Obesity; Multiple Sclerosis; Stereology.

POSTER PRESENTATION

ESTIMATION OF TOTAL VENTRICULAR VOLUME AND VOLUME FRACTION OF BRAIN IN NORMAL CT SCANS - A STEREOLOGICAL STUDY

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Introduction: The study of ventricular volume has recently become a centre of attraction in some neuropsychiatric diseases like Schizophrenia, Alzheimer's disease and Chronic Alcoholism. Volumetric analysis of ventricular system is also helpful in the diagnosis and classification of hydrocephalus and in the assessment, follow-up during its therapy (ventricular shunts). Some of these diseases also present with brain parenchymal atrophy leading to ventricle/brain ratio changes. This baseline study was done to determine the total ventricular volume and ventricle-total brain volume fraction related to different gender and age groups in normal adult population.

Material and Method: 50 cranial non-contrast computed tomography (CT) scans of normal adult subjects (29 females and 21 males) were collected from the museum of department of Anatomy, Maulana Azad Medical College, New Delhi. The total ventricular volume and volume fraction of total ventricular volume to total brain volume were estimated using the Cavalieri method and volume fraction-stereological methods. Statistical analysis was done using the independent t-test in SPSS version 23.

Result: The difference in mean total brain ventricle volume was statistically significant between the two genders (27.55 cm³ in males and 22.34 cm³ females). Mean volume fraction of total ventricular volume to total brain volume was found to be 2.77% in males and 2.25% in females which was also significant. Both mean ventricular volume and volume fraction was found to increase with age.

Conclusion: We calculated a normal baseline data of total ventricular volume and volume fraction of total ventricle to total brain in adult population by stereological tools from simple CT scans.

Keywords: Ventricular volume, Alzheimer's disease, hydrocephalus, Stereology.

AN INSIGHT INTO SOME PRELIMINARY STEROLOGICAL PRINCIPLES ADHERED TO IN THE EVALUATION OF RAT LIVER HISTOMORPHOMETRY AFTER SUCRALOSE INGESTION

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Introduction: Stereology provides meaningful quantitative descriptions of the geometry of 3D structures from measurements that are made on 2D images. Stereological rules for measurements allows for unbiased quantification in toxicological pathology. In the present study, Size of hepatocytes, Size of nuclei of hepatocytes and Sinusoidal width was measured in two groups of rats and compared with each other. Stereological aspects related to the study were highlighted.

Material and method: Sampling principles followed were:

1. Selection of animals: Inbred Adult Wistar albino rats were procured from Animal lab, UCMS and GTBH of weight 150-200 gms of either sex. 6 experimental rats were given 3 gm/kg/day sucralose dissolved in distilled water with an oral gavage whereas control rats were given equal amount of distilled water.
2. Selection of sample site: 4 pieces each having thickness 5 mm were taken for paraffin sectioning from 4 lobes of rat liver, namely left, Middle, Right and Caudate lobe.
3. Sample size: 100 slides were made from each liver taking very 7th serial section and stained with either Hematoxylin and Eosin (80 slides), Periodic acid Schiff (10 slides) and Masson's Trichrome stain (10 slides).
4. Histomorphometric measurements were done for individual rat liver and checked for similar changes in all the 4 pieces of livers taken from different lobes.
5. Assessment of histomorphometric parameters was done taking stereological rules into consideration.

Result: The present study presents the basic stereological principles while assessing the histomorphometric changes in liver in Adult wistar albino rats after ingestion of pure sucralose.

MORPHOMETRY OF ISLETS OF LANGERHANS IN HUMAN FETUSES

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Background: Current research on diabetes and its treatment modalities relies majorly on the information obtained from rodent and non-primate models. However, such models cannot reflect the complexity of human islet cytoarchitecture, morphometry and pathophysiology. Research reveals that islet morphometry such as size of islets determines survival rate. Type 2 diabetes patients have a disproportionate loss of larger islets. Also, transplant of fetal pancreatic islet cells show better results than adult islets cells. Hence, information regarding islet morphometry in human fetus is of utmost importance. This study was therefore, aimed at exploring certain parameters as regards islets in the developing human embryos at various gestational ages.

Materials and methods: This study was conducted on 10 human foetuses ranging from gestational ages 14 week to 36 week obtained from department of obstetrics and gynaecology, Lok Nayak hospital after written informed consent from parents. After formalin fixation and tissue processing for paraffin embedding, serial sections of fetal pancreas were generated and stained with haematoxylin and eosin stain and immunohistochemistry for B cells was performed using anti insulin antibody. Morphometry was conducted using an image pro plus software attached to a BX61 Olympus microscope fitted with DAP 71 Olympus camera. The mean diameter and mean area of the islets as well as the numerical density of islets was calculated separately for head, body and tail region.

Observations: The mean area of the islets in tail region was $108.12\mu\text{m}^2$ at 14 weeks. At 36 weeks the mean area of the islets in head region was only $224.66\mu\text{m}^2$ while in the tail region it was $817.75\mu\text{m}^2$. The numerical density ranged from 1.73×10^{-6} per μm^3 at 14 weeks to 3.71×10^{-6} per μm^3 in the tail. Increase in numerical density of islets in tail region was slow uptill 24 weeks followed by a sudden spurt beyond this age. In the body region, the density showed a gradual increase from 14 to 36 weeks.

Conclusion: This study reveals various morphometric details about the fetal islets. We need more of such data to understand the human fetal islets and thereby contribute to better success rates in human fetal islets transplantation.

QUANTIFYING MASTOID PARAMETERS FOR SEXUAL DIMORPHISM – A RADIOGRAPHIC STUDY

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Aim : The objective of this study was to determine sexual dimorphism in the dimensions of the mastoid triangle in lateral radiographs of skulls and ranking their discrimination accuracy by employing Discriminant Functional Analysis.

Material and Methods : A total of 150 lateral radiographs of North Indian individuals from LLR Hospital, Kanpur with known sex and ages; 80 men and 70 women were studied using DICOM image analyzer software.

Result : The values of measurements i.e mastoid length = 30.22 ± 5.61 mm for males and 25.98 for females; Porion- Mastoidale 32.21 for male, 31.66 mm for female. Mastoidale- asterion 50.00 mm male, 49.84 mm female. Asterion-Porion 44.11 mm male 39.72 mm female. The results of the triangle calculations in males and females which is 690.74 ± 23.35 mm² and 570.57 ± 130 mm² respectively.

Conclusion : All the dimensions of the mastoid triangle measured larger in males than females and were statistically significant. All variables analyzed together showed the highest accuracy. (90%) in classifying the sex followed by mastoid length (88%) next Area Mastoid triangle (78%) Mastoidale-asterion length (75.2%) Asterion-porion (70.1%) porion- mastoidale (69.0%)

Key Words: Sexual dimorphism; Mastoid process; Lateral radiographs.

DETERMINATION OF SEX USING CLAVICLE MEASUREMENTS: A DISCRIMINANT ANALYSIS

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Introduction : Establishment of the identity of the deceased from its skeletal remains is a difficult problem and becomes even more challenging when only a single bone is available. This study reported sexual dimorphism of human clavicle in order to create population specific discriminant tools for morphometric sex assessment.

Material and Method : The studied sample consisted of 160 adult clavicles retrieved from the Department of Anatomy, Pt. B.D Sharma PGIMS, Rohtak. The specimens were well documented and represented North Indian population of surrounding zone. Length, weight , volume and mid-clavicular circumference of the clavicles was recorded.

Results : Multivariate discriminant functional analysis involving length, weight and mid-clavicular circumference could correctly identify 68% of male clavicles of both sides. However, only 46.7% of female clavicles of both sides could be identified accurately by this combination.

Conclusion : Males exhibited a higher percentage of sex predictability on various combinations of morphometric parameters as compared to females.

Keywords : Sex determination, clavicle, discriminant function analysis, morphometric parameters.

MORPHOMETRIC ANALYSIS OF TYPICAL THORACIC SPINE IN INDIAN POPULATION AND ITS SURGICAL IMPLICATIONS

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Background: The frequent surgical interventions of thoracic spine are more common due to a wide array of traumatic, degenerative and neoplastic diseases. For successful surgical management of these conditions, a detailed anatomical knowledge of the thoracic spine is required.

Aim: The aim of this study was to present a morphometric reference database for thoracic vertebrae of the Indian population.

Material and methods: 250 dry human typical (T2-T8) thoracic vertebrae of undetermined gender and age were selected from the bone bank of the Department of Anatomy, Maulana Azad Medical College New Delhi. Linear measurements of the vertebrae were taken with the help of digital Vernier caliper and angular measurements were determined with software image J. The mean and standard deviation of the linear and angular parameters were calculated.

Results: The anterior and posterior height of the body was 17.60 ± 1.32 mm and 19.27 ± 1.64 mm respectively. The vertebral body width was 25.59 ± 2.49 mm. The antero-posterior distance of the vertebral body was found to be 21.78 ± 3.26 mm and transverse diameter of the vertebral body was 27.22 ± 2.06 mm. The A.P distance of vertebral canal (APD) was 14.29 ± 1.18 mm. The Pedicle height (PH) was 11.15mm, The Pedicle length (PL) was 6.65 ± 1.06 mm, The Interpedicular distance (IPD) was 15.52 ± 1.30 mm, The Cord length (C.L) was found to be 30.53 ± 3.03 mm. The transverse pedicle angle (TPA) was 13.06 ± 3.06 degrees. The Sagittal pedicle angle (SPA) was 15.52 ± 1.30 degrees and the Spinous process angle (SPRA) was 59.82 ± 8.66 degree.

Conclusion: The results provide a comprehensive database for more accurate modelling and design of vertebral body implants and instrumentations for Indian population.

Key Words: Pedicle width, Pedicle angle, Chord length, Sagittal pedicle angle, Spinous process angle.

ABSENCE OF RIGHT HORIZONTAL FISSURE AND LEFT OBLIQUE FISSURE IN A PAIR OF LUNGS – A CADAVERIC CASE REPORT

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Introduction : Situated on either sides of the heart and other structures in the mediastinum, and occupying the major part of the thorax, the pair of lungs act as the essential organ of respiration. Generally right lungs is divided into three lobes by one right horizontal and one right oblique fissure while the left lung is divided into two lobes by left oblique fissure. Variation in embryological origin may cause incompleteness or absence of fissures and thus changes in lobar arrangements.

Material & Method : During performing routine dissection for undergraduate students, this rare case was noticed in the lungs of a 58 years old formalin fixed female cadaver in the Department of Anatomy of Regional Institute of Medical Sciences, Imphal. The specimen was photographed and comparisons were made with normal ones.

Result : The right lung showed the presence of a single fissure. The oblique fissure was present and it divided the lung into two lobes namely upper and lower lobe. However, horizontal fissure was absent. In the left lung, there was absence of the oblique fissure. Costal surface and structures passing through hilum were normal in both sides.

Conclusion : The lung fissures enhance uniform expansion, and their position can be used as reliable landmarks in thoracic lesions. Surgically, the gradation of fissure is important in order to approach the ligation of vessels and bronchi through the depth of the fissure. Knowledge of anatomical variation in fissures is important for segmental resections, lobectomies and radiological reporting of lung pathologies.

CYSTIC HYGROMA WITH HYDROPS FETALIS - A CASE REPORT

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Introduction : Cystic hygromas are malformations of the lymphatic system that appear as fluid filled, thin walled, multiseptate cystic structures posterior to the fetal head and neck eccentrically situated with respect to the long axis of the fetus without vertebral column defect. They result from the jugular lymphatic obstruction sequence in which the normal communication between the jugular veins and the jugular lymphatic sacs fail to develop by 40th day of gestation. If a connection between the lymphatic and venous system does not occur at this point, a progressive peripheral lymphoedema and hydrops develops leading to early uterine death. Cystic hygroma has a strong association with turner's syndrome.

Material & Method : An abnormal fetus was collected from the department of obstetrics and gynaecology, RIMS, Imphal after taking permission from concerned authorities and parents. The maternal history was collected and the fetus was further evaluated.

Result : On examination, it was a female fetus showing generalised swelling of the body with a large swelling in the head and neck region. The fetus also showed facial dysmorphic features like flattened nasal bridge and low set ears.

Conclusion : The survival rate of fetuses affected with cystic hygroma is only 2-6% when diagnosed in utero. Once diagnosed, identifying associated anomalies and assessment of development of fetal hydrops should be of prime concern. Genetic counselling to the patient's family is necessary to discuss the need for karyotyping, potential risks to the fetus and risks in subsequent pregnancies.