ISSN: 0970-1842

JAS

Journal of Anatomical Sciences

PUBLISHED BY : UP CHAPTER OF ANATOMICAL SOCIETY OF INDIA

Editor-in-Chief

Dr. Navneet Kumar

Volume: 25(1), June 2017

www.asiup.in

JOURNAL OF ANATOMICAL SCIENCES

General Information:

The Journal is published twice a year.

The journal publishes original and unpublished research communications written in English in the form of full length papers and proceedings of the conference.

Subscription Rate:

Individual Suscription: Rs. 1000 per annum.

Institutional of Library Subscription : 2000 per annum.

Information for subscribers: The order should be placed with the Editor : Dr. Navneet Kumar, Department of Anatomy, King George's Medical University, Lucknow-226003 (India)

Email: editorjasup@gmail.com | Mob. 9415083580

The order should accompany an advance remittance by Ban Draft/Cheque in favour of Editor JAS, Allahabad Bank, KGMC, Lucknow

Back Volume: Information for the price and availability of back volumes can be obtained from Editor.

JOURNAL OF ANATOMICAL SCIENCES
INSTRUCTIONS TO AUTHORS

The journal publishes- 1. Full length papers, 2. Brief communications including case reports, 3. Review articles, 4. Book reeviews and 5. Scientific proceedings of U.P. Chapter of the Anatomical Society of India.

The full length papers and brief communications should report original researches in the fields of Anatomy, including Gross Anatomy, Comparative Anatomy, Embryology, Histology, Histochemistry, Cytogenetics, Radiological Anatomy, and Allied Clinical Medicine.

CONDITIONS FOR SUBMISSION

The manuscripts written in British English typed in double space, on side of the paper with a wide margin around, submitted in duplicate (with two sets of illustrations), are reviewed for publication, with the explicit understanding that they are being submitted with one journal at a time and have not been published in or accepted for publication elsewhere, completely or in part. It is author's responsibility to obtain permission to reproduce illustrations, tables etc. from other publications. The accepted papers will become permanent property of the Journal of Anatomical Sciences, and may not be reproduced in whole or in part, by any means, without the written consent of both, the author and the publisher.

The editor reserves the right to improve on the style and grammar and make corrections accordingly and, if necessary, return the manuscript for revision to the authors.

ARRANGEMENT OF THE MANUSCRIPT

The manuscript should have a uniform style and should consist of the following subdivisions, and in that order, each to begin on a separate page. Title page, Abstract and Key words, Text, References, Explanation to the figures, Tables and illustrations.

Title Page: The first page of every manuscript should include 1. Title of the article. 2. Full Name(s) of the author(s) 3. Institutional address of each author 4. Institution or the laboratory where the work was conducted. 5. A short running title. 6. Address for correspondence.

Abstract: Should be on the second page of each manuscript, not exceeding 300 words. The abstract should include brief introduction, objectives, main results and important conclusions.

Key Words: A list of 3-9 key words should be given below the abstract.

Text: The test of the manuscript should include 1. Introduction, 2. Material and Methods, 3. Observations, 4. Discussion, 5. Conclusion, if any, in that order.

References: References should be arranged according to VANCOUVER SYSTEM on the following indications.

The references No. should be in the order of appearance in the text and denoted in brackets example [1] etc.

Papers published in periodicles: 1. Each author's surname followed by initials 2. Full title of the paper 3. Abbreviation of the journal according to the style of index medicus 4. Year of Publication 5. Volume number, followed by a colon 6. First and the last page of paper. For example **Longia GS, Kumar V, Gupta CD. Intra renal arterial pattern of human kidney – corrosion cast study. Ant Anz. 1982; 166:183-194.** Work referred from the books: 1. Each Editor's surname followed by initials 2. Full title of book 3. Name of Chapter 4. Edition 5. Name of publisher 6. Domicile of publisher 7. Year of publcation 8. Page number (a small 'p' with a full stop should be prefixed to the page number , pp with a full stop if the number of the pages are more. For example: **Snell RS. Clinical Anatomy By Regions. The Perineum 9th ed. Philadelphia: Lippncott Williams & Wilkins; 2014, pp. 314-315**.

In the text, references should be made by author's surname, if there are one or two authors, followed by the year of publication in brackets, for example: Style (1966) or Longia and Kumar (1982). If there are more than two authors, mention first author's surname followed by et al. and then the year of publication in brackets, for example Singh et al. (1980). However, in

the list of References names of all the authors as indicated in the para above, should be given.

Full Address: At the end of the manuscript, after the list of references, exact postal address of the main author who will handle the reprints and the correspondence, should be given complete in all respects together with the postal PIN code. Any change in the address should be immediately communicated to the Editor.

Tables: All the tables should be referred to in the text and should be numbered in Roman numerals. Every table should be prepared on a separate sheet and should have a heading (complete and brief) and may have footnotes. The tables should be simple with as few as possible vertical and horizontal lines. Too long and exhaustive tables are not acceptable because they cannot be reproduced as such.

Illustration: All the illustrations including charts and diagrams and graphs should be referred to in the test and should be numbered. Every illustration should have legend typed on a separate page. For good reproduction, while printing, only good drawings and original photographs will be accepted. Negatives or photocopies of figures and illustrations are not acceptable. A size greater than 120 mm x 150 mm will necessitate reduction for reproduction must be submitted in complete and finished form with adequate labeling. The photographs should be made on glossy papers and should be provided with the legends. The abbreviations used in each illustratioin should be arranged alphabetically and should be included with the respective legends. All the illustrations should have on the reverse, written with light pencil, figure number, name of author(s) and the top indicated by the arrow. While dispatching they should be well protected to avoid postal/transit damage.

Authors will have to pay for planning scanning, plate making & printing on art paper @ Rs. 500/- per Black & White, @ Rs. 1000/- per coloured photograph & @ 500/- per table, graph & drawn figures. If the total amount of the bill is less than Rs. 1000/- It will be considered rounded off to theminimum payment of Rs. 2500/-.

One one copy of the Journal will be provided to the author.

REMITTANCE

Bank Draft only drawn in favour of EDITOR, JOURNAL OF ANATOMICAL SCIENCES, payable at Lucknow.

For transfer of Fund

Acc/No. : 50383876885

IFSC Code : ALLA0211028

MICR Code : 226010013

Note:

1. Strict compliance of instructions is mandatory failing which article will be rejected and no correspondence in this matter will be entertained. Send Three (3) laminated envelopes of requisite size with postage stamps affixed to cover registered AD Post for the articles to be sent to refrees/advisors. The manuscript in a CD (with Figures & Tables) should also be included.

ADDRESS FOR CORRESPONDENCE

Dr. Navneet Kumar (Professor)
Editor JAS
Department of Anatomy,
King George's Medical University,
Lucknow-226 003
Mob. 9415083580
email:editorjasup@gmail.com
navneetchauhan@hotmail.com

Dr. Archana Rani (Professor)
Joint Editor JAS
Department of Anatomy,
King George's Medical University,
Lucknow-226 003
Mob. 9451950799
email: editorjasup@gmail.com
archana71gupta@yahoo.co.in

OFFICE BEARERS & EXECUTIVE COMMITTEE MEMBERS OF U.P. CHAPTER OF ASI

President	:	Dr. Naresh Chandra Goel (Barabanki)
Vice Presidents	:	Dr. Satyam Khare (Meerut) Dr. Rakesh Kumar Diwan (KGMU, Lucknow)
Secretary cum Treasurer	:	Dr. Kuldeep Singh (Badhaun)
Joint Secretary cum Joint Treasurer	:	Dr. Shubha Srivastava (Meerut)
Editor	:	Dr.Navneet Kumar (KGMU, Lucknow)
Joint Editor	:	Dr. Archana Rani (KGMU, Lucknow)

Executive Members: Dr. Rakesh Gupta (Bareilly), Dr. Ranjeet Kumar (Barabanki), Dr. Pramod Kumar (Jalaun), Dr. Alok Singh (Jalaun), Dr. Alka Singh (Mayo, Lucknow), Dr. Badal Singh (Allahabad), Dr. Aditya Singh (Varanasi), Dr. M.K. Pant (Dehradun), Dr. Khayati Sant Ram (Chandigarh), Dr. Shweta (Barabanki).

Membership Fees:

Ordinary Membership	:	Rs. 500/-
Life Membership	:	Rs. 4000/-

SECRETARIAT
D-109, Shastri Nagar, Meerut-250004
Ph. 9720052244
E-mail: dr_kuldeep68@yahoo.com

C O N T E N T S

ORIGINAL ARTICLES

THE JOURNAL IS INDEXED IN

- Index copernicus International (ICV 2016: 66.02)
- Scopemed
- Index Scholar
- Google scholar
- Indian Science Abstract

Original Article

MORPHOMETRIC STUDY OF HYPOGLOSSAL CANAL AND ITS ANATOMICAL VARIANT IN NORTH INDIAN SKULLS

Priyanka Sinha*, Neeraj Kumar*, Nripendra Singh, Anupriya Kumar***
*Department of Anatomy, TSM Medical College and Hospital, Lucknow, UP, India.
**Department of Community Medicine, TSM Medical College and Hospital, Lucknow

ABSTRACT

Introduction: Hypoglossal canal transmits hypoglossal nerve, ascending pharyngeal artery, and an emissary vein. Occasionally it is divided by bony spicule leading to an anatomical variant. Taking into consideration the clinical condition and congenital defects, and to avoid damage during surgical intervention, knowledge of morphometry of this region is important.

Material & Methods: Forty dry adult human skulls, 20 male and 20 female, were taken from the Department of Anatomy, TSM Medical College, Lucknow. Skulls were observed for any damage of posterior cranial fossa and only those with good condition were selected. For morphometric analysis, we used Digital Vernier caliper to measure the anteroposterior and transverse diameter of extracranial opening of hypoglossal canal. Presence or absence of double hypoglossal canal was also observed.

Results: The anteroposterior and transverse diameter of hypoglossal canal was more in males than in females. Unilateral double hypoglossal canal was noted in 15% dry skulls whereas no bilateral double hypoglossal canal was observed in this study.

Keywords: Hypoglossal canal, skull, variant.

INTRODUCTION

The vertebrate skull is most modified part of axial skeleton and is adapted to protect the brain, special senses and cranial nerves [1]. The study of base of the skull and its foramina provides information about the evolutionary history of man. The hypoglossal (anterior condylar) canal is a paired bone passage that is located on occipital condyle and runs lateral to and slightly forward from the posterior cranial fossa to the nasopharyngeal carotid space and transmits the hypoglossal nerve. The hypoglossal canal (HC) is occasionally marked by presence of bony septum; this division of the HC support the basioccipital bone theory, which states that the formation of basioccipital bone is by the union of 3 or 4 previously separate vertebrae. According to this theory, the HC reflects the union of the intervertebral foramen [2,3].

The hypoglossal canal contains hypoglossal nerve, which originates from the hypoglossal nucleus in the brain stem as a number of small rootlets that extends through the medulla oblongata in a paramedian location (intra-axial segment). The rootlets then merge together to form the hypoglossal nerve, exiting the hypoglossal canal of the occipital bone (skull base segment). Beside the hypoglossal nerve, the canal also contains venous plexus, emissary vein and a branch of the ascending pharyngeal artery [4-9]. The venous plexus is an important content of this canal as it creates a link between the marginal sinuses and the superior jugular bulb and, indirectly, with the vertebral veins [10,11]. Occasionally the canal is divided by bony spicule leading to a variant called as

Address for Correspondence:
Dr. Priyanka Sinha, Assistant Professor, Department of Anatomy, T.S. Mishra Medical College & Hospital, Lucknow. UP ,India.
Mob. 8795173579 | Email:priyankasinha971@gmail.com

double hypoglossal canal. Berry (1975) studied this cranial variant on racial basis, which is of importance for anthropologist [12].

Taking into consideration the conditions such as fracture of the occipital bone, intra-cranial and extracranial neoplasm, hypoglossal canal lesions and congenital defects, and to avoid damage during surgical intervention such as transcondylar, supracondylar and lateral suboccipital approach, knowledge of morphometry of this region is important. Therefore, the present study was undertaken to measure the dimensions of canal, and to record the incidence of anatomical variant like double hypoglossal canal.

MATERIAL AND METHODS

Forty dry adult human skulls, 20 male and 20 female, were taken from the Department of Anatomy, TSM Medical College, Lucknow. Skulls were observed for any damage of posterior cranial fossa and those in good condition were selected. For morphometric analysis, we used Digital Vernier caliper to measure the anteroposterior and transverse diameter of extracranial opening of hypoglossal canal. Single and presence or absence of double hypoglossal canal was also observed (Fig.1,2). The readings were analyzed statistically for mean and standard deviation. The data obtained were used for statistical analysis by student's t test. The level of significance was $p < 0.05$.

Fig. 1: Photograph showing single hypoglossal canal

Fig. 2: Photograph showing double hypoglossal canal

OBSERVATIONS AND RESULTS

In males, the mean anteroposterior diameter on right side was 0.89cm and on left side was 0.85cm.In females, it was 0.79cm on right side and 0.78cm on left side. Average anteroposterior diameter on right side was 0.84 cm and on left side was 0.82cm (Table1).

Table 1: Analysis of A-P diameter (cm) of hypoglossal canal

A.P. DIAMETER						
	Male		Female		Average	
	Right	Left	Right	Left	Right	Left
RANGE	0.42 - 1.34	0.46 - 1.31	0.51 - 1.15	0.52 - 1.23	0.42 - 1.34	0.46 - 1.31
MEAN	0.89	0.85	0. 79	0.78	0.84	0.82
P value	>0.05		>0.05		>0.05	

Mean transverse diameter in males was 0.7cm both on right side and left side, while in females it was 0.6cm both on right side and left side. Average transverse diameter on right side was 0.66cm and on left side was 0.65cm (Table 2).

Table 2: Analysis of transverse diameter (cm) of hypoglossal canal

TRANSVERSE DIAMETER						
	Male		Female		Average	
	Right	Left	Right	Left	Right	Left
RANGE	0.37-1.23	0.30-1.91	0.33-1.21	0.28-1.27	0.33-1.23	0.28-1.91
MEAN	0.7	0.7	0. 6	0.6	0.7	0.6
P value	>0.05		>0.05		>0.05	

On application of student t test, statistically significant difference were observed between male and female skulls regarding both anteroposterior and transverse diameter with p value of<0.05.

Incidence of double hypoglossal canal was also observed and we found that out of 40 skulls (20 male and 20 female), only 6 skulls (4 male and 2 female) had unilateral double hypoglossal canal which accounts for 15% of skulls. Out of 6 skulls, in 2 skulls (1 male and 1 female) it was present on left side and in 4 skulls (3 male and 1 female) it was present on right side. No bilateral double hypoglossal canal was observed in this study.

DISCUSSION

The precise location and dimensions of the HC is important in understanding of the spatial relationships of surrounding structures. This knowledge is important for radiologists and neurosurgeons for the resection of tumors of posterior cranial fossa like schwannoma of hypoglossal nerve and sleep apnoea treatment. In addition, when drilling into the Occipital Condyle it is important for the surgeon to anticipate the possible depth and direction of the HC [12].

Berge & Bergman (2001) showed that AP diameter and transverse breadth of hypoglossal canal was 0.55x0.42 cm [13]. Berlin et al. (1992) recorded the morphometry of hypoglossal canal as 0.9x0.5 cm [14]. Sharma & Garud (2011) found that on right side was it was 0.53x0.43 cm and on left side was 0.54x0.43 cm [15]. In the present study, the mean anteroposterior diameter and transverse diameter of the HC in males on right side was 0.89x0.7cm and on left side was 0.85x0.7cm whereas in females we found the mean anteroposterior diameter and transverse diameter of the HC on right side 0.79x0.6cm and on left side 0.78x0.6cm respectively.

Cranial variants like incidence of double hypoglossal canal were studied by many workers for utilising this trait as anthropological marker. Berry & Berry (1967) studied the hypoglossal canal in different population of the world and found that the frequency of such separation was noted in 7-27.4% [16]. Zaidi et al. 92011) observed the incidence of double hypoglossal canal in North Indian crania as 12.5% [17]. Wysocki et al. (2004) found in 43% of skulls [18]. In present study, 15% of skulls showed the presence of double hypoglossal canal.

CONCLUSION

The dimensions of hypoglossal canal are important for neurosurgeons for planning skull base surgeries. Study of cranial variant like incidence of double canal has importance for anthropologist and forensic experts. In present study, the anteroposterior diameter and transverse diameter was more in males than females. There was no significant difference between right and left side values. Unilateral double hypoglossal canal was observed in 15% of skulls, more on right side. No bilateral canal was observed in this study. Thus the present study adds on to the data on morphometry of hypoglossal canal of North Indian skulls and compares with data of different regions of the world.

REFERENCES

1. Nikumbh RD, Nikumbh DB, Karambelkar RR, Shewale AD. Morphological Study of Hypoglossal Canal and Its Anatomical Variation. IJHSR. 2013;3(6):54-58.
2. Bastianini A, Guidotti A, Hauser G, et al. Variations in the method of the division of the hypoglossal canal in Sienese skulls of known age and sex. Acta Anat. 1985;123:21–24.
3. Hauser G, de Stefano GF. Variations in form of the hypoglossal canal. Am J Phys Anthropol. 1985;67:7–11.
4. Lang J, Hornung G. The hypoglossal channel and its contents in the posterolateral access to the petroclival area. Neurochirurgia. 1993;36: 75–80.

5. Leblanc A. Les nerfscrâniens. Springer, Berlin, Heidelberg, 1995;260–268.
6. Reinhardt KR, Miller ME, Evans HE. The craniovertebral veins and sinuses of the dog. Am J Anat. 1962;111:67–87.
7. Schwaber MK, Netterville JL, Maciunas R. Microsurgical anatomy of the lower skullbase- a morphometric analysis. Am J Otol. 1990; 11:401–405.
8. Sullivan WS. The anatomy of the rhesus monkey. Williams, Baltimore. 1961.
9. Wysocki J, Kobryń H, Kobryńczuk F. Proportion analysis of hypoglossal canal elements in human, macacus and dog. Med Sci Monit. 1998; 4 (Suppl. 2): 52–54.
10. Gisel A. Die Venenim Canalis nervi hypoglossi. Zeitschrf Anat u Entwickl. 1956; 119: 257–258.
11. Katsuta T, Matsushima T, Wen HT, et al. Trajectory of the hypoglossal canal: significance for the transcondylar approach. Neurol Med Chir (Tokyo). 2000;40:206–210.
12. Berry AC. Factors affecting the incidence of non-metrical skeletal variants. J Anat. 1975;120 (3):519-535.
13. Berge JK, Bergman RA. Variations in size and in symmetry of foramina of the human skull. Clin Anat. 2001;14(6):406-413.
14. Berlin A, Putz R, Schumacher M. Direct CT measurements of canals and foramina of the skull base.Br J Radiol.1992;65:653-66.
15. Sharma NA, Garud RS. Morphometric evaluation and a report on the aberrations of the foramina in the intermediate region of the human cranial base: A study of an Indian population. Eur J Anat. 2011;15(3):140-149.
16. Berry AC, Berry RJ. Epigenic variation in the human cranium.J Anat. 1967;101:361-80.
17. Zaidi SHH, Gupta R, Usman NA. Study of hypoglossal canal in North Indian crania. J Anat Soc India. 2011;60(2):224-226.
18. Wysocki J, Kobryn H, Bubrowski M, Kwiatkowski J, Reymond J, Skarzynska B. The morphology of the hypoglossal canal and its size in relation to skull capacity in man and other species. Folia Morphol. 2004;63:11-17.

Original Article

HISTOPATHOLOGICAL CHANGES INDUCED BY DESVENLAFAXINE IN CEREBELLUM OF SWISS ALBINO MICE

Amrita Kumari*, Mandavi Singh, Anand Mishra****
*Department of Anatomy, Indira Gandhi Institute of Medical Sciences, Patna
**Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi

ABSTRACT

Introduction: Desvenlafaxine succinate is a novel salt form of isolated major active metabolite of venlafaxine. It has been classified as serotonin and nor-epinephrine reuptake inhibitor [SNRI] which increases their concentration in synaptic cleft by inhibiting reuptake pumps. The increased concentrations of these neurotransmitters are responsible for the effect of these drugs on brain and their anti-depressant property.

Methods: Desvenlafaxine was administered to adult female Swiss albino mice in the dose of 80mg per kg body wt. by oral gavage. According to the duration of exposure of drug, mice were divided into following three groups. Group 1 (control) were treated with equivalent amount of tap water. In Group 2, mice were given drug for the first 6 days of gestation while in Group 3, they were treated from gestation days 1-18.

Results: Cerebellum of group 2, showed alteration in the molecular layer in comparison to control. The laminar pattern was maintained but differentiating purkinje cells was not visible. Granular cell layer appeared to be more thickened as compared to control.

Cerebellum of group 3, showed marked derangement of laminar pattern. Extensive vacuolation extending upto molecular level was seen. Plenty of pyknotic nuclei were present.

Conclusion: Above changes suggest probable role of Desvenlafaxine in causing neurodegenerative and oxidative damage to cerebellum of Swiss albino mice.

Key words: Depression, serotonin, nor-epinephrine, purkinje cell.

INTRODUCTION

Desvenlafaxine succinate is chemically bicyclic phenylethylamine compound launched as a, sustained release formulation for the treatment of depression [1]. In vitro studies, suggest that it causes more potent blockage of norepinephrine transporter than parent drug [2]. It lacked significant affinity for muscarinic, cholinergic, H1-histaminergic or α_1-adrenergic receptors. In addition to that, it doesn't show affinity for various ion channels like calcium, chloride, potassium and sodium [3]. Desvenlafaxine demonstrates good brain to plasma ratio suggesting utility in CNS, peripheral nervous system and peripheral related disorders with change in neurotransmitters [2]. Studies of postpartum depression suggest a 10-15% incidence in all parturients, making it most common complications of pregnancy [4]. The depressive episodes in women also seem to increase in pregnancy so treatment become must. Although this drug seems to have lesser side-effects but well controlled studies suggesting its safety profile in pregnancy is missing so we have done this study to see the effect of Desvenlafaxine on brain tissue of foetus.

Address for Correspondence:
Dr. Amrita Kumari, Senior Resident, Department of Anatomy, IGIMS, PATNA
Email: amritakumariims@gmail.com

MATERIAL AND METHODS

The present study was done in the Department of Anatomy, Institute of Medical Sciences, BHU, Varanasi. After getting the Institutional Ethical Committee clearance, adult female Swiss albino mice were chosen for the study. Rats were divided in 3 groups. Group 1 served as control while groups 2 and 3 as experimental animals. The experimental animals were harboured in plastic polypropylene cages at a room temperature of 25^0C±2 and relative humidity around 50-60%.The mice were fed with pelleted diet and tap water was given ad libitum. Male and female mice in the ratio of 2:1 were kept for mating in the evening and the following morning female mice were examined for the presence of vaginal plugs which will indicate about positive pregnancy status of the female mice. Those vaginal plug positive female mice were given drug in the dose of 80 mg/kg body wt. from day 1-6 (Group 2) and day 1-18 of gestation (Group 3).The control female mice (Group 1) received equivalent amount of tap water for the corresponding period via same route. All the following group animals were reared and fetuses were delivered by uterotomy on 19^{th} day of gestation under deep ether anaesthesia. After fixation in neutral formalin, brains were removed for the study. The fixed brain tissue were processed, sectioned and stained with haematoxylin and eosin to study effect of Desvenlafaxine on microanatomy of the foetal brain.

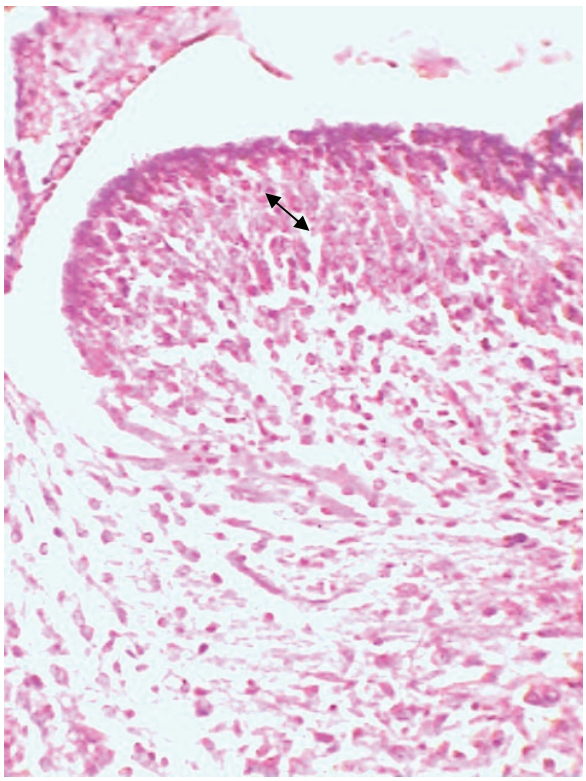

Fig. 1: Cerebellum of foetus of group 1(control) showing well differentiated layers and well differentiated purkinje cells [↘]

RESULTS

Although the drug doesn't seem to affect external morphology much, histological changes are suggestive of neurodegenerative changes induced by the drug.

In cerebellum of group 1 (Fig. 1) rounded cells were seen which were in the process of differentiation into purkinje cells. Cerebellum of group 2 (Fig.2) showed differentiated molecular and granular layers but these were thinner and less organized as compared to group 1 (controls). Such cells were still not differentiated in group 2. In group 3 (Fig.3) cerebellar cortex was markedly infiltrated with edematous spaces. The granular and molecular layers showed many degenerating cells. The thickened granular layer indicated less differentiation and migration of cells towards the surface. Differentiated purkinje cells were not observed. The cells of granular layer were enlarged in comparison to group 1 and 2 cells.

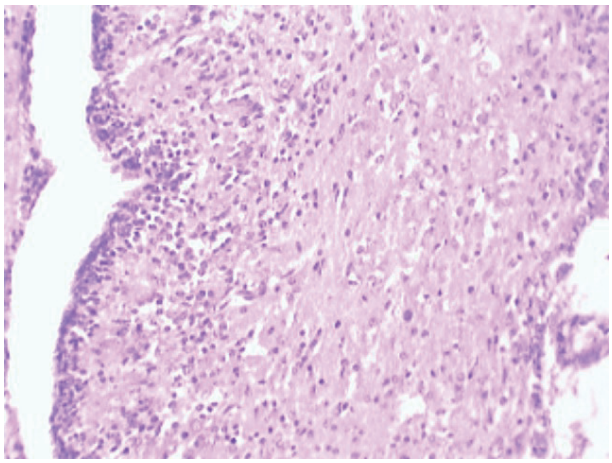

Fig.2: Cerebellum of group 2, showing only slight alteration in the molecular layer. The laminar pattern is maintained but differentiating purkinje cells are not visible. Granular cell layer seems to be thickened. (H&E X 400)

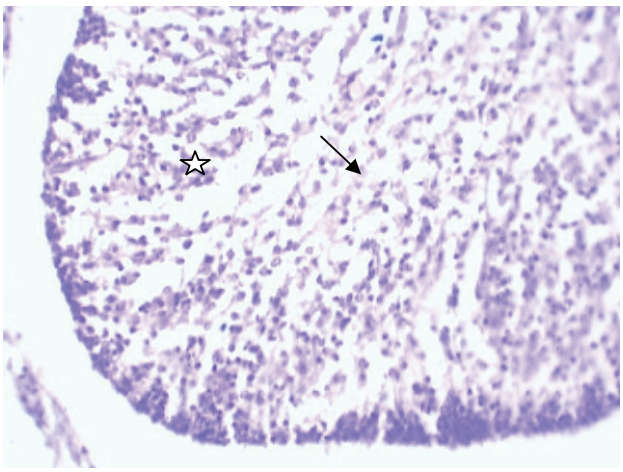

Fig.3: Cerebellum of foetus of group 3, showing marked derangement of laminar pattern. Extensive vacuolation [☆] extending upto molecular level can be seen. Plenty of pyknotic nuclei [↘] are seen (H&E X 400).

DISCUSSION

The lifetime risk for depression in women varies from 10 to 30% with peak prevalence during the childbearing years [5, 6]. Some anxiety disorders such as panic disorders may even worsen during pregnancy and requires pharmacological treatments [7]. Desvenlafaxine is the active metabolite of venlafaxine, acts as dual reuptake inhibitor of serotonin and norepinephrine. Although it is called as dual reuptake inhibitor, they have a third action on dopamine in the pre-frontal cortex. This third mechanism of boosting dopamine in brain actually proves its efficacy in the treatment of major depression. In our study, we observed histological changes in cerebellum as derangement in the laminar pattern with slight alteration in molecular layer. Extensive vacuolation can be seen. Desvenlafaxine is not metabolised by CYP2D6 and is excreted unchanged or after conjugation. It also has lower potential for drug interactions, so various unconjugated or un-metabolised oxidised products accumulation in the synaptic cleft and brain tissue seems to be the culprit for the various neurodegenerative changes as seen in various slides. Here by, the drug should be used judiciously specially in case of pregnant females.

REFERENCES

1. Schatzberg AF, Nemeroff CB. The American Psychiatric Publishing Textbook of Psychopharmacology, 4th edition, Chapter 22, 2009, pp. 511-521.
2. Deecher DC,Beyer CE,Johnston G, et al. Desvenlafaxine succinate: a new serotonin and norepinephrine reuptake inhibitor. J Pharmacol Exp Ther 2006; 318(2):657-665.
3. Pristiq (package insert). Philadwphia, PA:Wyeth Pharmaceutical; 2008.
4. Wisner KL, Perel JM. Psychopharmacological treatment during pregnancy and lactation. In: Jensvold MF, Halbreich U, Hamilton JA, editors. Psychopharmacology and Women: Sex, Gender and Hormones. Washinton DC: American Psychiatric press, 1996; pp.191-224.
5. Burke KC, Burke JD Jr, Rae DS, Regier DA. Comparing age at the onset of major depression and other psychiatric disorders by birth cohorts in five US community populations. Arch Gen Psychiatry. 1991; 48(9):789-95.
6. Kessler RG, McGonagle KA, Zhao S, et al. Lifetime and 12-month prevalence of DSM-III-R Psyciatric disorders in the United States. Results from national comorbidity survey. Arch Gen Psychiatry. 1994; 51:8-19.
7. Cohen LS, Sichel DA, Dimmock VA & Rosenbaum VF. Impact of pregnancy on panic disorder: a case series. Journal of clinical psychiatry. 1994; 55:282-292.

Original Article

EFFECT OF PRALLETHRIN VAPOURS ON CEREBELLAR CORTEX OF ALBINO RATS: A NEUROHISTOLOGICAL STUDY

Zeba Alam*, Azmi Mohsin, S.M.Yunus***, Farida Ahmad***, Nafis Ahmad Faruqi*****
*Anugrah Narayan Magadh Medical College, Gaya. (Bihar) India
**Major S. D. Singh Medical College & Hospital, Farrukhabad. (U.P.) India
***J. N. Medical College, AMU, Aligarh. (U.P.) India

ABSTRACT

Introduction: The Prallethrin is a synthetic form of natural Pyrethrin and due to their highly insecticidal properties has achieved widespread agricultural and environmental health applications. This is commonly used as mosquito repellent in most houses throughout the year, especially during night exposing the adults and children to its vapour.

Methods: Adult male Charles Foster rats were exposed to 3.2%w/v of prallethrin for 12hrs daily for a period of 90 days.

Results: Neurohistological examination showed that inhalation of Prallethrin based mosquito repellent can hamper the precise patterned arrangement of the cerebellar cortex.

Discussion: It was concluded that prolonged exposure to the prallethrin vapours are neurotoxic and one should be cautious, while using it.

Key words- Prallethrin, insecticide, cerebellar cortex,neurotoxicity.

INTRODUCTION

The Prallethrin is a form of Pyrethroids which are derivatives of natural Pyrethrins [1,2]. The latter are highly insecticidal, therefore considered for agricultural and environmental health applications. These are commonly used as mosquito repellent (MR) to protect humans against attack by mosquitoes. In developing countries, such repellents are widely used in most houses for years especially during nights. As a result, adults and children are exposed to the vapour of Pyrethroid containing mosquito repellent [3]. Male mice exposed to the smoke of mosquito coil containing D-allethrin have shown morphological changes in the respiratory system [4]. Short term exposure to bio-allethrin has been reported to cause irritation of eyes, skin and respiratory tract along with neurotoxic effects [5-7]. They demonstrated that the developing nervous system is especially vulnerable if exposed to MR during early stages of development (critical brain growth period). Most of the toxicological and physiological investigations concerning the effects of pyrethroids have been performed on adult animals receiving high doses and on in vitro systems using nerve membrane preparations [8-10]. Eriksson and Nordberg (1990) studied the neurotoxic effects of Pyerthroids in immature mouse brain [11]. There are also literatures pertaining to the adverse effects of Pyrethroid based mosquito repellent on infants causing significant abnormalities affecting the CNS by breaching blood brain barrier. With reports of increased incidence of Pyrethroid induced neurotoxicity and increased usage of Pyrethroid based compounds for household pest control, the study of their effect on general population becomes relevant, especially when there is growing evidence that indoor exposure to pesticide is worldwide [12-14]. It is interesting to note that there is higher pesticide concentration in urban compared to rural areas [15].

Address for Correspondence:
Dr. Zeba Alam, C/o Justice Mehfooz Alam, Nikhat Villa, Justice Lane, Pethiya bazar, Phulwari Sharif, (200 Yards West of Mahavir Cancer Sansthan)**,** Patna. (Pin Code – 801505) Bihar. India**,** Mob: 9572276048 **|** Email: zeba.shabbir@yahoo.com

Although there are several studies on the neurotoxic effects and the effects on functional integrity of developing blood brain barrier following exposure to pyrethroids, there are very few studies on the structural integrity of CNS after exposure to mosquito repellent containing pyrethroids. The aim of present study is to assess whether Pyrethroid based mosquito repellent can affect the cortical organization of the cerebellum on prolonged inhalation in adult albino rats.

MATERIAL AND METHODS

Adult male Charles Foster rats, weighing between 120-150 gm were used in this experiment. They were housed in unit plastic cages (36cm×22cm×14cm) and provided with standard pellet laboratory diet (LIPTON INDIA LIMITED) and water ad-libitum. The cages were placed in a closed room (180/240cm).The animals in experimental group were exposed to 3.2%w/v of prallethrin for 12hrs daily for a period of 90 days. The control animals were kept under ideal conditions without exposure to prallethrin vapours. The permission for the study was taken from Institutional Animals Ethics Committee, for using rats for study.

After 90 days of exposure the animals from both groups were anaesthetized and perfused. The brain was dissected out from dorsal aspects. Sections of 5µm thickness from cerebellar cortex were prepared for neurohistological examination. Three types of histological stains were used (a) Haematoxylin and eosin (b) Thionine (c) Luxol fast blue.

RESULTS

H&E stained sections of cerebellar cortex of the experimental animals showed decreased uptake of stain, increased thickness of molecular layer, malalignment of purkinje cells, increased density of cells in the granular layer and vacuoles in white matter as compared to well stained and well demarcated trilaminar cortex visualized in control animals (Fig 1 & 2).

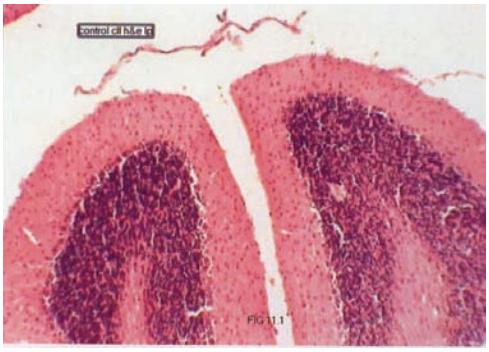

Fig. 1: Photomicrograph of cerebellar cortex of control rat (HE stain;100x)

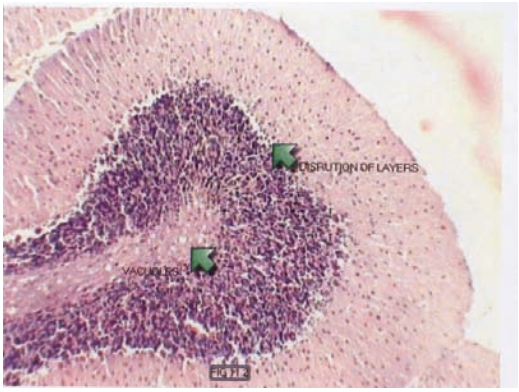

Fig. 2: Photomicrograph of cerebellar cortex of Prallethrin exposed rat (HE stain; 100x)

Luxol fast blue stained experimental cerebellar cortex showed numerous vacuoles as compared to the intact fibre bundle of the cerebellar cortex of the control animals (Fig 3 & 4).

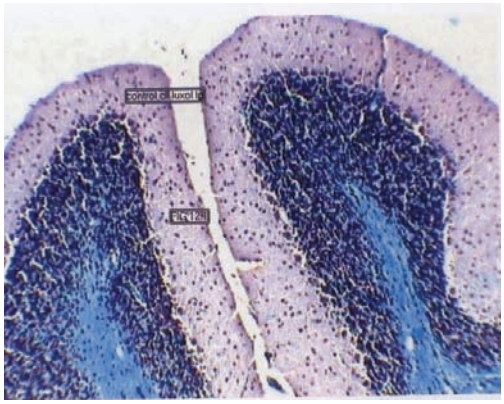

Fig. 3: Photomicrograph of cerebellar cortex of control rat (Luxol Fast stain; 100x)

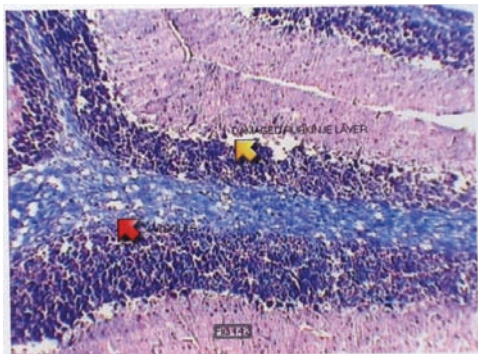

Fig. 4: Photomicrograph of cerebellar cortex of Prallethrin exposed rat (Luxol Fast stain 100x)

Thionin stained tissues of cerebellar cortex of experimental animal showed separation of molecular layer from purkinje cell and increased density of nuclei in granular layer (Fig 5). Same areas under higher magnification showed irregular spacing of purkinje and increase in the density of nuclei in granular layer and separation of molecular layer (Fig 6).

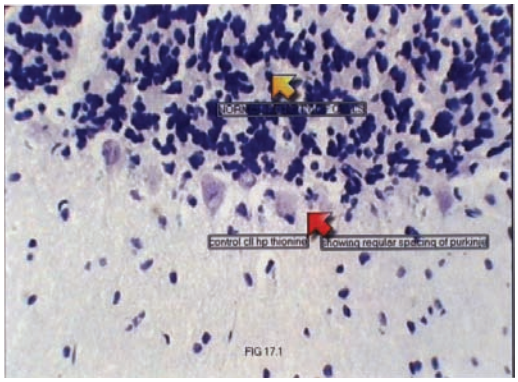

Fig. 5: Photomicrograph of cerebellar cortex of control rat (Thionin stain; 400x)

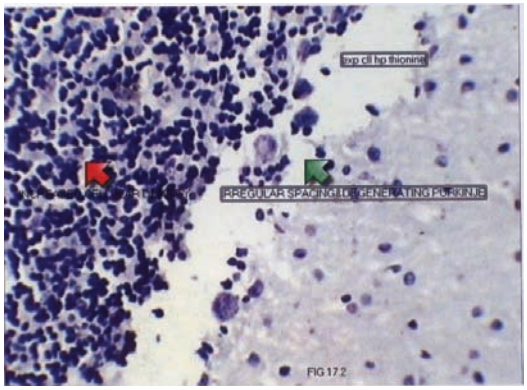

Fig. 6: Photomicrograph of cerebellar cortex of Prallethrin exposed rat (Thionin stain; 400x)

DISCUSSION

Pyrethroid compounds are being widely used as an efficient means of pest and vector control. Their widespread usage lead to their persistence and accumulation in the environment. Since environmental exposure to these widely used compounds is quite common and there is growing evidence that indoor pesticide exposure is of considerable magnitude pesticide concentration may be especially high in urban areas. As a result their usage and toxic profiles remain an issue of debate.

Pyrethroids are reported to prolong the sodium ion channel opening, thereby increasing in the sodium current inflow, which in turn may lead to hypertension and convulsion, followed by paralysis [16]. Post-exposure symptoms of pyrethroid compounds are tremor, choreoathetosis and salivation [11].

The studies conducted by Sinha et al. (2003) on developing albino rats showed the damage done by pyrethroids to the developing blood-brain-barrier [17]. Hence the present study was done to look for histological evidences, if any. Also to find out whether they are toxic only to developing brain or to adult brain too. The present study has shown that there were changes in the cerebellar cortex of adult male rat following exposure to 3.2% w/v of prallethrin vapours.

CONCLUSION

In the presence of such profound effects, evident in the histological sections there are possibilities of effect on behavior and learning i.e. psychomotor effect which may be associated with biochemical changes, which require further study.

ACKNOWLEDGEMENTS

We wish to thank Mr. Shahabuddin and Mrs Farhana Naqvi for their technical assistance in the preparation of the histological sections.

REFERENCES

1. Elliot M, Janes NF, Kimmel FC, et al. Metabolic fate of pyrethrin 1, pyrethrin 2 and allethrin administered orally rats. J Agric Food Chem. 1972; 20(2):300-313.
2. Elliot M. Properties and application of pyrethroid. Environ Health Perspect 1976; 14:3-13.

3. Gupta A, Nigam D, Gupta A, Shukla GS, Agarwal AK. Effect of pyrethroid-based liquid mosquito repellent inhalation on the blood-brain-barrier function and oxidative damage in selected organs of developing rats. J Appl Toxicol. 1999; 19:67-72.
4. Cheng V, Lee HR, Chen CS. Morphological changes in the respiratory system of mice after inhalation of mosquito-coil smoke. Toxicol Lett. 1992; 62:163-177.
5. Anon Bioallethrin. International Programme of Chemical safety (IPSC). Commission of European Communities. WHO. 2920 Luxenburg. Grand duchy of Luxenberg. Geneva 27, Switzerland., 1991, pp 1211.
6. Leng G, Kuhn KH, Wiesler B, Idel H. Metabolism of bioallethrins and related compounds in human. Toxicol Lett. 1999; 107:104-121.
7. Mehl A, Schanke TM, Jhonsen BA, Fonnum F. The effects of trichlorfon and other organophosphates on prenatal brain development in the guinea pig. Neurochem Rev. 1994; 19:569-574.
8. Casida JE, Gammon DW, Glickman AH, et al. Mechanisms of selective action of pyrethroid insecticides. Rev Pharmacol Toxicol 1983; 23:413-438.
9. Gray AJ, Soderlund DM. Mammalian toxicity of pyrethroids. In; insecticides. Hutson DH, Roberts TR, eds. New York, Wilcy., 1985; 193-248.
10. Narahashi T. Nerve membrane ionic channels as the primary target of pyrethroids. Neurotoxicol. 1985; 6(2):3-22.
11. Eriksson P, Nordberg A. Effects of two pyrethroids bioallethrin and deltamethrin, on subpopulations of muscarinic and nicotinic receptors in the neonatal mouse brain. Toxicol Appl Pharmacol. 1990; 102:456-463.
12. Lewis RG, Fortmann RC, Camann DE. Evaluation of methods for monitoring the potential exposure of children to the pesticides in the residential environment. Arch Environ Contam Toxicol. 1994; 26:37-46.
13. Whitemore RW, Immerman FW, Camman DE, Bond AE, Lewis RG, Schaun JL. Non-occupational exposure to pesticides for residents of two US cities. Arch Environ Contam Toxicol. 1994; 26:47-59.
14. Landrigan PJ, Claudio L, Markowitz SB, Berkowitz GS, Brennet BL. Pesticides and inter-city children exposure, risk and prevention. Environ Health Perspect. 1999; 107:431-437.
15. Adagate JL, Barr DB, Clayton CA, Eberly LE, Freeman NCG. Measurement of children's exposure to pesticides; analysis of urinary metabolite levels in a probability based sample. Environ Health Perspect. 2001; 109:586-590.
16. Vijverberg HPM, Van Den Bercken J. Neurotoxicological effects and the mode of action of pyrethroid insecticides. Crit Rev Toxicol. 1990; 21(2):105-126.
17. Sinha C, Agarwal AK, Islam F, Seth K, Chaturvedi RK, Shukla S, Seth PK. Mosquito repellent (pyrethroid based) induced dysfunction of blood-brain barrier permeability in developing brain. Int J Devl Neuroscience. 2004; 22:31-37.

Original Article

A STUDY OF WORMIAN BONES IN NORTH INDIAN POPULATION

R.K. Diwan*, R.K. Verma*, A.K. Pankaj*, Archana Rani*, Anita Rani* & Sushma Tomar*
* Department of Anatomy, King George's Medical University UP, Lucknow, India

ABSTRACT

Introduction: Wormian bones are a subset of the small intrasutural bones that lie between the cranial sutures formed by the bones of the skull vault. They are formed due to additional ossification centres in or near sutures. They vary in size, shape and number. Wormian bones are the accessory bones which are usually not present in all normal skulls. The aim of the present study was to see the incidence, number and location of these accessory bones in human dry skulls.

Material and Methods: The present study was carried out on 90 dry adult human skulls of unknown age and sex which were collected from osteology lab of Anatomy Department of King George's Medical University, Lucknow, UP. The skulls were cleaned, dried properly and were looked for the presence of wormian bones in respect to its location and number.

Observation & Results: In the present study, the incidence of Wormian bones was seen in 35 skulls (38.88%) out of 90 skulls. We found maximum number of Wormian bones at lambdoid suture i.e. 22 skulls (24.44%) on left side and 21 skulls (23.33%) on right side. We also found such bones at squamous suture i.e. 5 skulls (5.55%) on left side whereas 6 skulls (6.66%) on right side. It was observed at coronal suture in 1 skull (1.11%) on left side whereas 3 skulls (3.33%) on right side. Only one skull showed Wormian bones on sagittal suture bilaterally i.e. both on right (1.11%) and left side (1.11%). In our study, we didn't find any accessory bones at asterion, occipitomastoid suture and near bregma.

Conclusion: It is important to know about these bones because they can mislead in the diagnosis of fracture of skull bones. Radiologists and neurosurgeons should keep in mind about such occurrence of accessory bones before doing craniotomy surgeries.

Key words: Wormian bones, skull, suture, inca bone, fontanelle.

INTRODUCTION

Occipital ossification states that above the highest nuchal lines, the squama is developed in a fibrous membrane and ossified from two centres: one on each side at about the second fetal month; this part may remain separate as the interparietal bones, the rest is performed in cartilage. Below the highest nuchal lines, the squama ossifies from two centres, appearing in about the seventh week and soon uniting. These two regions of the squama unite in the third postnatal month but the line of union is recognizable at birth. An additional ossification centre may occur in or near sutures, giving rise to isolated sutural bones (also called Wormian bones). They are irregular in size and shape, and most frequent in the lambdoid suture, they also occur at fontanelles, especially the posterior fontanelle. They may represent a pre-interparietal element, a true interparietal, or a composite. An isolated bone at the lambda is sometimes referred to

Address for correspondence:
Dr. Rakesh Kumar Diwan, Associate Professor, Department of Anatomy, King George's Medical University UP, Lucknow, India-226003
Mob: 8005335622 | Email: dewanrakesh80@yahoo.com

as an Inca bone or Goethe's ossicle. Sutural bones usually have little morphological significance. However, they appear in great numbers in hydrocephalic skulls and they have therefore been linked with rapid cranial expansion [1]. The Wormian bones or Sutural bones or Ossicles are islands of small bones found at the sutures and fontanelles of the skull, most commonly in the lambdoid sutures [2]. The first description of sutural bones is attributed to Paracelsus (1460 to 1541 CE (Common Era) however they are named after the Danish anatomist, Olaus Wormius. The alternative names of Wormian Bones include Schaltknochen (Leichner-Weil, 1964), intercalary, sutural and intrasutural bones [3]. It is unclear at this time exactly how or why they are formed, although genetic as well as environmental factors have been proposed. Their initial formation is thought to be caused by a degree of dural strain and increased sutural width. These conditions can result from mechanically induced stress due to intentional deformation like that practiced in ancient cultures, premature sutural closure, or from reduced skull ossification as seen in metabolic bone diseases. The cause of the malformation can have an influence on the number and location of Wormian bones. Clinically, Wormian bones are used as markers in the diagnoses of many autosomal dominant genetic disorders, namely, craniosynostosis and osteogenesis imperfecta [4]. The aim of the present study was to see the incidence, number and location of these accessory bones in human dry skulls. It is important to know about these bones because they can mislead in the diagnosis of fracture of skull bones.

MATERIAL AND METHODS

The present study was conducted on 90 dried adult human skulls of unknown sex and origin obtained from the Anthropology lab of Anatomy Department of King George's Medical University UP, Lucknow. Each skull was closely observed for presence of Wormian bones at various sutures. Broken or damaged skulls were excluded from the study. The number (single, double, multiple) and location of these Wormian bones on human dry skulls was observed and the total incidence of Wormian bones on left and right side was calculated.

OBSERVATIONS AND RESULTS

Maximum number of Wormian bones was located at lambdoid suture both on right and left side. Single, double and multiple Wormian bones were also present, maximum at lambdoid suture followed by squamous, coronal and sagittal sutures (Table 1,2&3).

Table 1: Location and number of Wormian bones on left side of skull

Location	Single	Double	Multiple
Lambdoid	10	8	4
Squamous	5	0	1
Coronal	1	0	0
Sagittal	1	0	0

Table 2: Location and number of Wormian bones on right side of skull

Location	Single	Double	Multiple
Lambdoid	18	1	2
Squamous	6	1	0
Coronal	3	0	0
Sagittal	1	0	0

Table 3: Incidence of Wormian bones at different areas of skull (n=90)

Location	Left side	Right side	Total (%)
Lambdoid	22	21	43 (47.77 %)
Squamous	5	6	11 (12.22 %)
Coronal	1	3	4 (4.44 %)
Sagittal	1	1	2 (2.22 %)

In the present study, the incidence of Wormian bones was seen in 35 skulls (38.88%) out of 90 skulls. We found maximum number i.e. 43 (47.77%) of Wormian bones at lambdoid suture i.e. 22 (24.44%) on left side whereas 21 (23.33%) on right side (Fig.1). Eleven (11) skulls i.e. 12.22% depicted such bones at squamous suture i.e. 5 skulls (5.55%) on left side whereas 6 skulls (6.66%) on right side (Fig.2). Skulls showing Wormian bones at coronal suture were 4 (4.44%) in number i.e. 1 (1.11%) on the left side whereas 3 (3.33%) on the right side (Fig.3). Only one skull was having Wormian bones on sagittal suture both on the left and right side (Fig.4). In our study, we didn't find any accessory bones at asterion, occipitomastoid suture and near bregma.

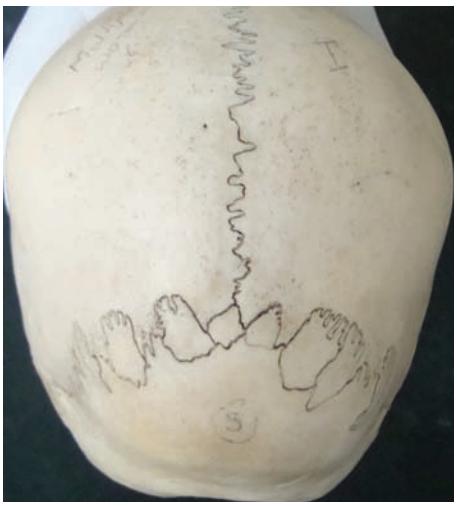

Fig. 1: Photograph showing Wormian bones on lambdoid suture

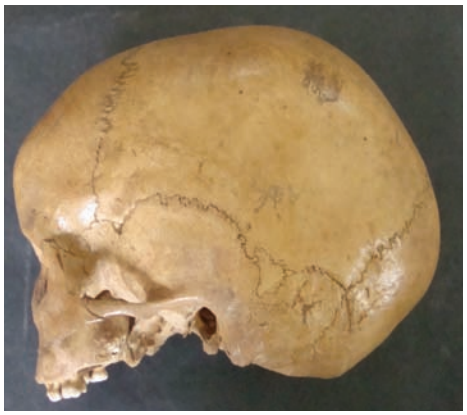

Fig. 2: Photograph showing Wormian bones on squamous suture

Fig. 3: Photograph showing Wormian bones on coronal suture

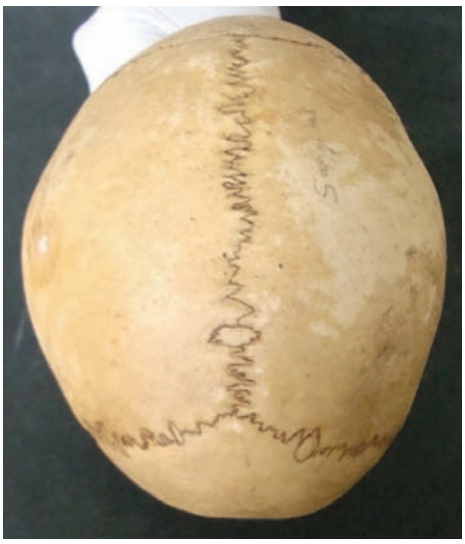

Fig. 4: Photograph showing Wormian bones on sagittal suture

DISCUSSION

Wormian bones are the normal variant present in the skull. The location of Wormian bones at different location has different importance. In the present study, overall incidence of Wormian bones was seen in 35 skulls out of 90 skulls. (38.88%). Pryles and Khan (1979) studied incidence and significance of Wormian bones (WBs) in a random group of infants and children. According to these authors, the prevalence of central nervous system abnormalities in a population with Wormian bones varies from 93% to 100% in a random group and reaches 100% in a mentally retarded population. Thus, the presence of WBs is almost invariably associated with abnormal development of the CNS and may serve as a useful marker for the early identification and treatment of the affected infant or child [5]. According to Bergman et al. (1988) nearly 40% of skulls have sutural bones in the vicinity of the lambdoid suture. The next most common site is the pterion ossicle, which has a high incidence among Indians. Although they are most commonly found in the posterior sutures (lamboidal and occipito-mastoid sutures), they can occur in any cranial suture and fontanelles [6]. Our study runs parallel with the findings of Bergman et al. as we also found most common incidence of Wormain bones at lambdoid suture (43%).

Wormian bones along the lambdoid suture are most common. The sutural bone at lambdoid suture is also termed as preinterparietal bone or inca bone. The

presence of series of Wormian bones may lead to problems in posterior approach to the cranial cavity. These bones might lead to confusion in interpretation of the radiographs in case of head injuries. The multiple Wormian bones may be mistaken for multiple fractures [7]. Brasili et al. [8] reported an incidence of 50.55% at lambdoid suture with more frequency among male skulls (p=0.649) while the present study showed total incidence of 47.77% with no sex difference. Along the squamous suture Brasili et al. [8] reported an incidence of 9.5% with more frequency among female skulls (Sessari sample) while the present study showed an incidence of 12.22% with no sex difference.

In the present study, the incidence of Wormian bones along the coronal suture was 4.44% with no sex difference while Brasili et al. [8] reported incidence of 3% with more frequency among male skulls. Brasili et al. [8] reported more frequency of Wormian bones along the sagittal suture in male skulls in Ozieri sample but in the present study, the incidence was 2.22% with no sex difference.

Lambda is the meeting point of lambdoid and sagittal suture and in infants it is called as posterior fontenelle and will be closed at 2-3 months after birth. Study of skulls showed a large number of sutural bones at the lambda and along the lambdoid suture [9]. Anton et al. (1992) hypothesized that such circumferential deformation placed compressional forces on the sutures resulting in fewer Wormian bones, while anteroposterior deformation increased tension across the sutures, thereby increasing the number of Wormian bones [10]. Along the course of life or during some medical emergencies we are in need to take the radiographs for analysis. During that period the x ray may show some extra bones in the skull other than the normal bones. There are possibilities for the clinicians to misinterpret it as a fracture rather than sutural bones [11].

CONCLUSION

The overall incidence of Wormian bones was 38.88%. The maximum incidence of Wormian bones at the lambdoid suture showed 47.77% whereas the minimum incidence observed at the sagittal suture was 2.22% on the basis of location and number.

REFERENCES

1. Williams PL, Warwick R, Dyson M, Bannister LH. Gray's Anatomy. 37th ed, London Churchill Livingstone.1989; 373, 387.
2. Sanchez-Lara PA, Graham Junior JM, Hing AV, Lee J, Cunningham M. The morphogenesis of Wormian bones: a study of craniosynostosis and purposeful cranial deformation. American Journal of Medical Genetics. 2007; 143A (24): 3243-3251.
3. Leichner-Weil, S. Das vorkommen yon schaltknochen in den schadelaufnahmen yon epileptikern. Radiologic Clinics of North America. 1964; 33:383-388.
4. Bellary SS, Steinberg A, Mirzavan N, Shirak M, Tubb RS, Cohen-Gadol AA, Loukas M. Wormian bones: a review. Clin. Anat. 2013; 26(8):922-7.
5. Pryles CV and Khan AJ. Wormian bones: A marker of CNS abnormality? American Journal of Diseases of Children. 1979; 133 (4): 380-382.
6. Bergman RA, Afifi AK, Miyauchi R. Skeletal systems: Cranium. In: Compendium of human anatomical variations. Baltimore, Urban and Schwarzenberg. 1988; 197-205.
7. Nayak SB. Multiple Wormian bones at the lambdoid suture in an Indian skull. Neuroanatomy. 2008; 7: 52-53.
8. Brasili P, Zaccagni L, Gualdi-Russo E. Scoring of nonmetric cranial traits: a population study. J Anat 1999; 195:551-562.
9. Seema PV, Mahajan A. Human skull with complete metopic suture and multiple sutural bones at lambdoid suture -a case report. International Journal of Anatomical Variations. 2014; 7: 7-9.
10. Anton SC, Jaslow CR, Swartz SM. Sutural complexity in artificially deformed human (Homo sapiens) crania. J Morphol. 1992; 214: 321–332.
11. Dumitriu DI, Menten R, Clapuyt P. Pitfalls in the diagnosis of common benign bone tumours in children. Insights into imaging. 2014; 5:(6):645-655.

Original Article

MORPHOMETRIC STUDY OF THIRD VENTRICLE WIDTH IN NORTH INDIAN POPULATION: A CT STUDY

Manjari Lohani[*], Garima Sehgal[**], Samir Kumar Mishra[***], PK Sharma[****], Neera Kohli[*****]
Department of Anatomy, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, Uttar Pradesh.
**Department of Anatomy, King George's Medical University, Lucknow, Uttar Pradesh.
***Charak Hospital, Lucknow.
****Department of Anatomy, Era's Lucknow Medical College & Hospital, Lucknow, Uttar Pradesh.
*****Department of Radiodiagnosis, King George's Medical University, Lucknow, Uttar Pradesh

ABSTRACT

Introduction: Brain is one of the most complex and magnificent organs in the human body. Ventricular system of brain comprises of two lateral ventricles, third ventricle and a fourth ventricle. As brain ages, there is a decrease in size of brain tissue accompanied by ventricular system enlargement along with increased cerebrospinal fluid volume. Ventricular enlargement also takes place in hydrocephalus and neurodegenerative conditions like Huntington's disease. It is very important to distinguish the pathological ventricular enlargement from age related ventricular enlargement. Keeping this in mind we endeavored to contribute a database for normal width of third ventricle in North Indian population.

Material and methods: A prospective study was conducted, whereby, radiologically normal CT scans of head region, belonging to 100 patients were subjected to thorough analysis in Radiant DICOM Viewer Software. The study was a joint venture between the Department of Anatomy and Department of Radiodiagnosis, King George's Medical University, Lucknow. The width of third ventricle taken at the level of interventricular foramen was measured on axial scans as the maximum distance between the two thalami. The study involved 51 males and 49 females (n=100) normal subjects ranging from 18 to 76 years, that were grouped into different categories according to age; 18-30 years, 31-40 years, 41-50 years, 51-60 years and above 60 years.

Results: Width of third ventricle was found ranging from 0 mm (trace/not measurable) to 8.4 mm with a mean of 3.62 mm and displayed a significant increase with increasing age. It was wider in males (3.92±2.42 mm) than females (3.30±1.72 mm). Width was found to be minimal and difficult to visualize in persons aged less than 35 years.

Conclusion: Knowledge about the mean width in different age groups and in either gender may help in diagnosis of some neurological disorders below the age of 49 years.

Keywords: Brain, third ventricle, CT scan, ageing, ventriculomegaly

INTRODUCTION

Human brain is one of the most complex and magnificent organs in the body. As brain ages, expected and characteristic structural changes occur which are considered normal. According to Coffey et al. (2001), healthy ageing in humans is generally associated with a decreased size of brain tissue, increased cerebrospinal fluid volume and ventricular enlargement [1]. The ventricular system comprises of a set of adjoining cavities that produce and circulate CSF within the brain and consists of two lateral

Address for correspondence:
Dr. Garima Sehgal, Assistant Professor, Department of Anatomy, King George's Medical University, Lucknow, Uttar Pradesh.
Mob: 9044513758 | Email: garimasehgal@kgmcindia.edu

ventricles, third ventricle and a fourth ventricle. Third ventricle is the cavity of diencephalon that lies in the midline between the right and left thalami. It communicates with each lateral ventricle via interventricular foramen and is continuous caudally with the cerebral aqueduct that leads to the fourth ventricle [2]. Understanding the normal and abnormal anatomy of the ventricular system of brain is helpful for clinicians, neurosurgeons and radiologists in their day to day practice.

Third ventricle is related to important brain structures including anterior commissure, fornix, tela choroidea, pineal body, hypothalamic structures, optic chiasma, infundibulum, mammillary bodies and tegmentum of midbrain [2]. Structural abnormalities involving any of the aforesaid regions can obstruct third ventricle and thus may cause hydrocephalus in infants or raised intracranial pressure in adults. Blockage of cerebral aqueduct may also lead to widening of third ventricle. Ventricular enlargement may also be associated with many neuro-degenerative disorders. Increased in pressure in third ventricle can cause hypothalamic symptoms like diabetes insipidus and obesity.

Various methods utilized to study the ventricular system of brain include pneumoencephalography, ultrasonography, luminal cast plastination method, CT and MRI studies.

We require a normal range of ventricular dimensions in order to report size of the ventricle as abnormal. The study was undertaken with the specific purpose of obtaining a data regarding normal width of third ventricle in various age groups and to find out any gender disparity if present.

MATERIAL AND METHODS

A cross-sectional study was conducted in Department of Anatomy, in collaboration with Department of Radiodiagnosis, using cranial CT scans obtained from Department of Radiodiagnosis, King George's Medical University, Lucknow, UP. The study group comprised of referred patients reporting for CT scan of head region due to various indications. CT scans reported radiologically normal in terms of cerebral ventricles and brain parenchyma were randomly collected in a DVD for analysis.

The study involved 100 normal subjects (51 males and 49 females) from 18 to 76 years, categorized into various groups; 18-30 years, 31-40 years, 41-50 years and 51-60 years and above 60 years. CT scan images of head region belonging to study subjects were procured from the Department of Radiodiagnosis. Approval was sought from the Ethics committee of King George's Medical University before the study started.

All CT scans were performed on 64-slice multidetector spiral CT scanner (Brilliance CT, Philips medical system, Nederland, B.V.5684 PC Best, The Nederlands) by trained and experienced staff under standardized conditions. CT was done in axial transverse scanning and optimum patient preparation and positioning was taken care of. After obtaining the view in lateral projection (120kVp; 30mA) orbito-meatal line was drawn and a line was drawn at an angle of 15 - 20 degrees to and 1 cm above it, representing the lowest tomographic section, which passed through the base of skull. The sections were taken with slice thickness of 5mm and increment of 10mm. Images were reconstructed at slice thickness of 1.25mm without any overlap.

CT scan images were collected in the DVD with relevant patient information. Analysis of digital images was done on personal laptop, using software tool Radiant DICOM Viewer (64-bit). The measurement was calibrated to nearer 0.1 mm. All images were studied in axial projection from below upwards.

Third ventricle was identified as a mid-line structure between right and left thalami. Width of third ventricle was measured in mm (millimeters) at the level of interventricular foramen (foramen of Monro) and was taken as the maximum distance between the two thalami at this level. The length of the third ventricle could not be measured as its posterior marker the pineal gland was not seen in CT scan sections of all patients.

Data obtained was analyzed statistically to obtain range, mean, standard deviation (SD). Student t' test was done to compare two groups and Analysis of Variance (ANOVA) to compare more than two groups at a time. P-value was calculated and p-value □0.05 was taken as significant.

OBSERVATIONS AND RESULTS

Width of third ventricle of normal human brain was measured on tomograms with respect to age and gender of the study population. Maximum width of third ventricle was found to be in the range of 0 mm (not measurable) to 8.4 mm (Fig. 1 & 2) with a mean of 3.62 mm and displayed a significant increase with increasing age (p-value □0.001) (Table 1).

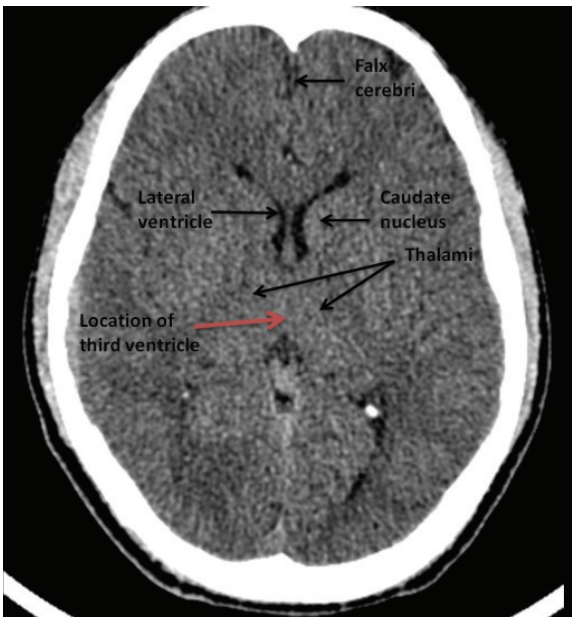

Fig. 1: Non Contrast CT Axial Image at the level of interventricular foramen showing trace third ventricle

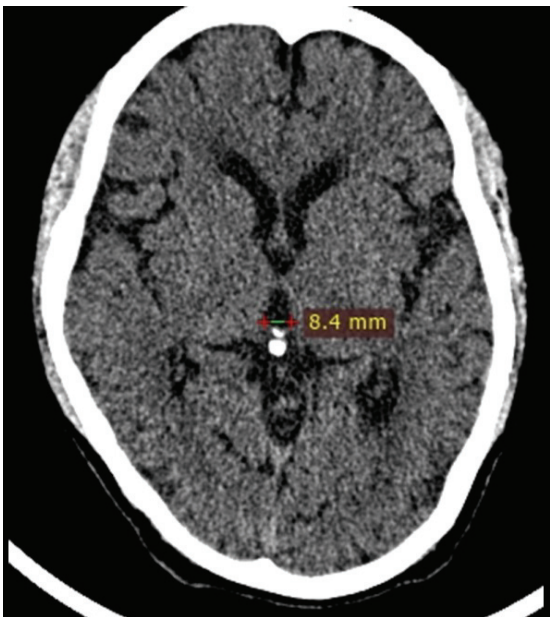

Fig. 2: Non Contrast CT Axial Image at the level of interventricular foramen showing maximum width of third ventricle

Table 1: Range of third ventricle width in various age groups

Age (years)	N=	Minimum (mm)	Maximum (mm)	Mean (mm)	SD (mm)
18-30	41	0	5.9	2.40	1.46
31-40	22	0	8.8	3.55	2.33
41-50	22	2	8.3	4.37	1.91
51-60	6	4.6	6.4	5.75	0.71
>60	9	4.8	7.8	6.04	1.17

Visualization of third ventricle width in persons aged less than 35 years was difficult and third ventricle width was found significantly lower in individuals between 18-30 years as compared to 40 years (Table 2).

We compared third ventricle width between males and females amongst various age groups, and found that third ventricle was wider in males (3.92±2.42 mm) than females (3.30±1.72 mm), though not statistically significant. Ventricular width increased with increasing age in both males and females. In the age group of 18-30 years, mean ventricular width was 2.31 mm in males and 2.51 mm in females. It was 4.56 mm in males and 2.85 mm in females in subjects between 31-40 yrs. We observed that in the age group of 41-50 years, the width measured 5.16 mm in males and 3.92 mm in females whereas it was 5.94 mm in males and 5.92 mm in females in the age group of 51-60 years (Table 2)

Mean width of third ventricle increased from 2.31 mm to 5.94 mm in males and from 2.51 mm to 5.92 mm in females as the age progressed (Table 2).

Maximum width of third ventricle was more in males than females for all the age groups except between 18-30 years where it was more in females (2.51±1.38 mm) than males (2.31±1.55 mm) though not statistically significant. No gender comparisons could be done for age group 51-60 years as there was no female in this age group (Table 2).

Table 2: Width of third ventricle in males and females of various age groups

Age (years)	Males			Females			Significance of difference	
	N=	Mean	SD	n	Mean	SD	't'	'p'
18-30	23	2.31	1.55	18	2.51	1.38	-0.41	0.681
31-40	9	4.56	3.09	13	2.85	1.36	1.77	0.093
41-50	8	5.16	2.33	14	3.92	1.54	1.51	0.147
51-60	6	5.75	0.71	0	0	0	-	-
>60	5	5.94	1.25	4	5.92	1.23	-0.283	0.785

DISCUSSION

Present study analyzed tomograms of 100 patients that were reported radiologically normal; and maximum width of third ventricle of brain was evaluated with respect to age and gender in the North Indian population.

Third ventricle is a narrow slit like cavity between two thalami. Normal size of the ventricles on CT and MRI has been found to be <5 mm in children, <7 mm in adults <60 years of age and <9 mm in adults above 60 years [3].

Investigators have employed various methods for measuring dimensions of third ventricle (Table 3). Borgersen (1966) in a postmortem study of ventricles stated that third ventricle is seen as trace/none before fourth decade [4]. Brinkman et al. (1981) conducted a CT study and compared ventricular dimension of 28 patients with Alzheimer dementia with that of 30 elderly persons with no history of neurologic disease. He found maximum width of third ventricle as 5.9 mm and also reported greater ventricular width in normal males [5]. Soininen et al. (1982) in a CT study on 57 patients with Alzheimer dementia, 19 patients with multi-infarct dementia and 85 controls of similar age and sex noted maximum width of third ventricle as 9.2mm in normal controls with higher values in males [6]. Le May M (1984) evaluated CT scans of 140 normal subjects ranging from 20-100 years and found none/trace of third ventricle in 16 persons in 20-29 years age group as compared to only 1 in 60-69 years. Mean width of third ventricle was reported between 0-4 mm [7].

In our study, we observed third ventricle width ranging between 0-8.4 mm (mean=3.62±2.12 mm). Ventricular width was found to be 2.4 mm among subjects who were 18-30 years of age, and it displayed an incremental pattern reaching 6.04 in >60 years of age. In 8 out of 100 subjects (18-33 years), width was observed as minimal/trace/none (0 mm), this was in confirmation to the findings of Borgersen [4] and Le May [7] who also stated that third ventricle is seen as trace/none before fourth decade. There was a significant difference in third ventricle width across different age groups (p=0.001). Mean width of third ventricle was found to be greater in males (3.92±2.42 mm) than females (3.30±1.72 mm) which was found to be statistically insignificant (p=0.148). Width of third ventricle was more in males than females across all age groups except in persons >60 years where it was found to be larger in females (6.18±1.23 mm) than males (5.94±1.25 mm). Our findings were in conformation to those of Brinkman et al. (1981) who also reported higher values in males [5].

Maximum width of ventricle was 8.4 mm in our study which was nearly similar to that reported by Kohlmeyer & Shamena (8.7mm) [8]. Though our study reports relatively higher third ventricle width as compared to many previous studies [9-12], our results were none the less similar to studies conducted by other authors [13, 14]. In addition, our findings differed from others who have reported still higher values for third ventricle width in contrast to our finding [15-18,]. Basis of this difference in dimensions could be firstly due to difference in the methods of study and measurement; luminal cast plastination , MRI, CT etc.; or secondly, the selection of study population (demography, age group etc.) amongst different studies. Mean width of third ventricle as reported by us is 2.40 mm in 18-30 yrs, 3.55 mm in 31-40 yrs, 4.37 mm in 41-50 yrs, 5.75 mm in 51-60 years and 6.04 mm in subjects more than 60 yrs. We found a consistent age related increment in the ventricular

width. Age related increase in the mean width of third ventricle as found in our study has also been reported previously in other studies. Our findings of a positive correlation between the third ventricle width and age is in agreement with the findings of Walhovd et al. (2005), who also reported significant age-related differences in all neuroanatomical volumes, including the thalamus and the third ventricle. Such an increase in width with increasing age may be independent of method of study or the demographic profile of study population [19].

Table 3: Comparison of studies on width of third ventricle

Authors	Study population	Method	n (M+F)	Age	Mean (mm)	SD (mm)	Range (mm)
Gawler et al. (1976)	London	CT scan	78	8 months-73 years			3-6
Gyldensted et al. (1977)	Denmark	CT scan	100 (50+50)	17-86 years			3.2-5
Haug (1977)	German	CT scan	170 (78+92)	0-75 years			2.4-5.4
Kohlmeyer et al. (1983)	German	CT scan	300	60-90 years			5.4-8.7
Duffner et al. (2003)	German	MRI	30		3.3	1.8	1.8-5.7
D'souza et al. (2007)	Goa	CT scan	1000 (500+500)	30-50 years	4.2		
Zauhair et al. (2009)	Baghdad	CT scan	112 (66+46)	10-69 years	2.28	0.61	
Meshram et al. (2012)	Indian	CT scan	112	21-60+ years	7.2	0.23	
Satapara et al. (2014)	Indian (Gujarat)	Plastination and MRI	83		5.2		
Patnaik et al. (2015)	Indian	CT scan	60	5-70 years	6.86	2.74	
Gameraddin et al. (2015)	Arab	CT scan	152 (89+63)	3-81 years	5.55	1.61	
Present Study (2016)	Indian (Uttar Pradesh)	CT scan	100 (51+49)	18-76 years	3.62	2.12	0-8.4

n: size of study population; M: number of males; F: number of females

CONCLUSION

Though ventricular enlargement is seen in many neurological disorders it is not a specific finding after the age 49 years since it is common in normal elderly. Nonetheless, knowledge about the mean width in different age groups and in either gender may help in diagnosis of some neurological disorders below the age of 49 years.

REFERENCES

1. Coffey CE, Ratcliff G, Saxton JA, Bryan RN, Fried LP, Lucke JF. Cognitive correlates of human brain ageing: a quantitative magnetic resonance imaging. J Neuropsychiatry Clin Neurosci. 2001;13(4):471-85.

2. Standring S, Healy JC, Johnson D, Borley NR, Collins P, Crossman AR et al. Gray's Anatomy, The Anatomical basis of clinical practice. 40th ed. Edinburgh: Elsevier Churchill Livingstone; 2008:227,237-240.
3. Meese W, Kluge W, Grumme T, Hopfenmuller W. CT evaluation of the CSF spaces of healthy persons. Neuroradiology. 1980; 19(3):131-6.
4. Borgersen D. Width of the third ventricle. Acta Radiologica (Stockholm). 1966; 4:645-62.
5. Brinkman SD, Sarwar M, Levin HS, Morris HH 3rd. Quantitative indexes of computed tomography in dementia and normal aging. Radiology. 1981; 138(1):89-92.
6. Soininen M, Puranen M, Reikkinen PJ. CT findings in senile dementia and normal ageing. J Neurol Neurosurg Psychiatry. 1982; 45: 50-54.
7. Le May M. Radiologic Changes of the Aging Brain and Skull. AJNR. 1984;5:269-275.
8. Kohlmeyer K, Shamena AR. Assessment of CSF spaces in the brain in demented and nondemented patients over 60 years of age. AJNR. 1983;4:706-7.
9. Gawler J, DuBoulay GH, Bull JHD, Marshall J. Computerized tomography: comparison with pneumoencephalography and ventriculography. J Neurol Neurosurg Psychiatry. 1976; 39: 203-11.
10. Gyldensted C. Measurements of the normal ventricular system and hemispheric sulci of 100 adults with computed tomography. Neuroradiology. 1977; 14:183-192.
11. Haug G. Age and sex dependence of the size of normal ventricles on computed tomography. Neuroradiology. 1977; 14:201-204.
12. Duffner F, Schiffbaur H, Glemser D, Skalei M, Freudenstein D. Anatomy of the cerebral ventricular system for endoscopic neurosurgery: a magnetic resonance study. Acta Neurochirgica (Wien). 2003; 145(5):359-68.
13. D'Souza DMC, Natekar PE. Morphometric study of the ventricular system of Brain by Computerised Tomography. J Anat Soc India. 2007; 56(1):19-24.
14. Zauhair A, Jaumah Fadhil A, AL-Kafhaji. CT Scan Measurements of the Lateral and Third Ventricles in Apparently Normal Iraqi Subjects. J Fac Med Baghdad. 2009; 51(3):320-322.
15. Meshram P, Hattangdi S. The morphometric study of third ventricle and diencephalon by computerized tomography. Indian journal of applied basic medical science. 2012;14(19):8-13.
16. Satapara VK, Patel MM, Rathava JK, Trivedi PN, Kukadiya UC, Gohil DV et al. Morphometry of width of third ventricle of brain by luminal cast Plastination & MRI. International Journal of Advanced Research. 2014; 2(6):837-842.
17. Patnaik P, Singh V, Singh S, Singh D. Gender Related Differences in Third Ventricle Parameters with Correlation to Cerebrum Size - A Study on Head CT Scans. International Journal of Health Sciences & Research. 2015; 5(11):140-147.
18. Gameraddin M, Alsayed A, Ali A, Al-Raddadi M. Morphometric Analysis of the Brain Ventricles in Normal Subjects Using Computerized Tomography. Open Journal of Radiology. 2015;5:13-19.
19. Walhovd, K. B., Fjell, A. M., Reinvang, I., Lundervold, A., Dale, A. M., Eilertsen, D. E., et al. Effects of age on volumes of cortex, white matter and subcortical structures. Neurobiol. Aging. 2005; 26: 1261–1270.

Original Article

INCIDENCE OF NUMERICAL CHROMOSOMAL ANOMALIES IN PATIENTS OF ACUTE MYELOID LEUKEMIA

Rakesh Kumar Verma*, Sangharsh Rao, Navneet Kumar*, Archana Rani* & Archana Kumar*****
*Department of Anatomy, King George's Medical University UP, Lucknow, India
**Department of Anesthesia, BRD Medical College, Gorakhpur, UP, India
***Department of Pediatrics, King George's Medical University UP, Lucknow, India

ABSTRACT

Introduction: Acute myeloid leukemia (AML) is a tumor of hematopoietic progenitors caused by acquired oncogenic mutations that impede differentiation, leading to the accumulation of immature myeloid blasts in the marrow. The single most important prognostic factor in AML is cytogenetics, which determine the prognosis and probability of relapse after treatment. Hence the cytogenetic analysis of AML patients plays a great role in prognosis and treatment.

Material and methods: Karyogram of diagnosed patients of AML was prepared from bone marrow and peripheral blood. This study was conducted in the Cytogenetic Laboratory of the Department of Anatomy, King George's Medical University UP, Lucknow. Patients were screened in the Department of Pediatrics Medicine and samples were collected from the Pathology Department.

Observations and Results: We observed the frequency of chromosomal aberrations in different age groups and sex. Out of 22 successful cases 12 cases (54.54%) exhibited abnormal karyogram and 10 cases (45.45%) showed normal karyogram. Numerical chromosomal abnormality was observed in 31.81% cases. Most common abnormality observed was trisomy 21 (13.63%) followed by trisomy 8 (9.09%) and trisomy 11 associated with trisomy 21 (4.54%).

Conclusion: Trisomy 21 was found to be most prevalent in UP region as compared to trisomy 8 and trisomy 11 associated with trisomy 21. Trisomy 21 has intermediate risk for developing AML, trisomy 8 is likely to be a disease modulating secondary event and trisomy 11 has poor prognostic value in AML patients. Hence the cytogenetic analysis of AML patients plays a great role in prognosis and treatment.

Key words: Acute myeloid leukemia (AML), karyogram, chromosomal aberrations, anomalies, trisomy

INTRODUCTION

Acute myeloid leukemia (AML is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal myoblast cells that accumulate in the bone marrow and interfere with the production of normal blood cells. AML is characterized by accumulation of cells at the early stages of the differentiation process [1].

AML is a heterogeneous disease, which comprises multiple subtypes [2,3]. The subtypes are classified according to the FAB classification system which is based on types and maturation of myoblast cells. Several risk factors and chromosomal abnormalities have been identified, but the specific cause is not clear. The single most important cause of AML is cytogenetic, or the chromosomal structure of the leukemic cell and also has prognostic value.

Address for correspondence:
Dr. Rakesh Kumar Verma, Assistant Professor, Department of Anatomy, King George's Medical University UP, Lucknow, India-226003
Mob: 9455252213Email: rakesh_gsvm@yahoo.co.in

Certain cytogenetic abnormalities are associated with very good outcomes (e.g., the (15; 17) translocation in acute promyelocytic leukemia). About half of AML patients have "normal" karyogram; they fall into an intermediate risk group. A number of other cytogenetic abnormalities are known to be associated with a poor prognosis and a high risk of relapse after treatment [4].

In AML, both numerical and structural chromosome aberrations have been shown to provide information about the clinical course. In many AML clinical trials, especially numerical chromosome aberrations like losses of chromosomes 5 and 7 as well as complex aberrations were identified as adverse prognostic factors for survival [5].

About 45-50% of AML patients have no detectable chromosomal abnormalities. In general, these individuals with a normal karyotype in their leukemic cells show an intermediate prognosis [6,7].

Hence cytogenetic analysis of AML patients plays a great role in diagnosis, explaining prognosis and deciding the treatment.

MATERIAL AND METHODS

This study was conducted in the Cytogenetic Laboratory of the Department of Anatomy, King George's Medical University, UP, Lucknow. Patients were screened in the Department of Pediatrics and Department of Medicine and the sample was collected from the Department of Pathology. The study was approved by ethical committee of King George's Medical University, UP, Lucknow. Karyogram was prepared from bone marrow and peripheral blood. The patients included in this study were clinically diagnosed cases (male or female) of any age group and informed consent was taken from each patient. Patients without consent (lack of consent) were excluded from the study.

OBSERVATIONS AND RESULTS

We observed the frequency of chromosomal aberrations in different age groups and sex. Total 34 cases were analyzed in which 22 karyograms were obtained successfully. Out of 22 successful cases, 12 cases (54.54%) exhibited abnormal karyogram and 10 cases (45.45%) showed normal karyogram. Numerical chromosomal abnormality was observed in 31.81% cases. Most common abnormality observed was trisomy 21 (13.63%) followed by trisomy 8 (9.09%) and trisomy 11 associated with trisomy 21 (4.54%).

Out of 34 patients, 22 (64.70%) were males and 12 (35.29 %) were females ranging from 1 year to 60 years of age. The ratio of male to female was 1.8:1. Out of which, 25 cases (73.52%) were in the age group up to 10 years & 9 cases (26.47%) were more than 10 years of age (Table 1, Fig. 1).

Table 1: Distribution of acute myeloid leukemia in different age groups

Age	No. of cases	% (n=34)
Upto 10 years	25	73.53%
More than 10 years	9	26.47%

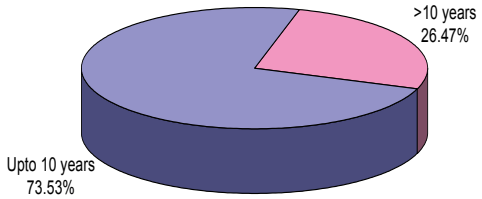

Fig. 1: Pie diagram showing distribution of acute myeloid leukemia in different age group

In successfully obtained 22 karyogram, 12 (54.54%) were male and 10 (45.45%) were female. Among 10 female karyogram, 6 karyogram were normal without any chromosomal aberrations and 4 karyogram were abnormal with chromosomal abnormalities (Table 2, Fig. 2).

Table 2: Gender wise distribution of cases with chromosomal abnormalities

Sex	Number of Cases	Karyogram Obtained	Normal Karyogram	Abnormal Karyogram
MALE	22	12	4	8
FEMALE	12	10	6	4
	Total= 34	Total =22	Total =10	Total =12

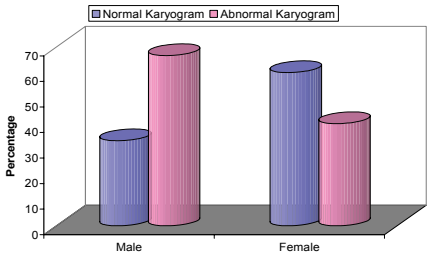

Fig. 2: Bar diagram showing gender wise distribution of cases with chromosomal abnormalities

In total 22 cases, numerical abnormalities were observed in 7 patients (31.81%). Trisomy 21 was found in 3 cases (13.63%). trisomy 8 in 2 cases (9.09%) and trisomy 11 in one case (4.54%) along with monosomy of 8 in one case (4.54%) only (Table 3, Fig. 3).

Table 3: Prevalence of numerical chromosomal abnormalities in overall studied karyograms/total cases with abnormal karyograms

Numerical Chromosomal Abnormalities	Number of Patients	% Karyogram of Obtained Cases (N= 22)
+21	3	13.63
+8	2	9.09
+11	1	4.54
-8	1	4.54
Total	7	

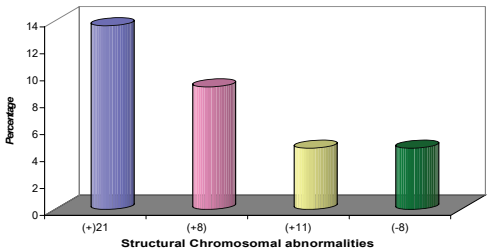

Fig. 3: Bar diagram showing prevalence of numerical chromosomal abnormalities in overall studied karyograms/ total cases with abnormal karyograms

According to age and gender, the different patterns of karyogram were found in different age groups and sex. 47XY, +21 karyogram (Fig. 4) was seen in 2 males of age 8 and 22 years, while one female aged 2 years was showing 48XX,+11,+21 (Fig. 5) type of karyogram. Out of this, the male patient was also showing monosomy of chromosome no. 8 and addition of p arm on chromosome no. 19. Trisomy 8 (Fig. 6) was found in 2 cases both were female aged 6 years & 26 years.

Fig. 4: Photograph of karyogram with 47, XY, +21 pattern

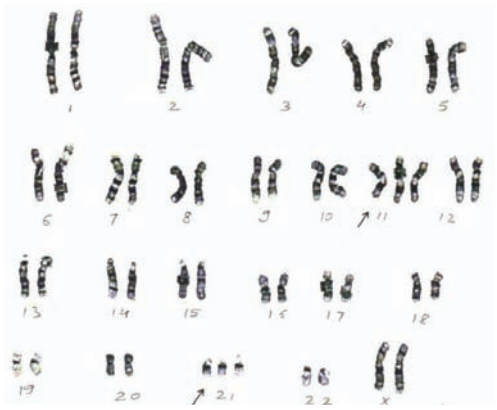

Fig. 5: Photograph of karyogram with 48, XX, +11, +21 pattern

Fig. 6: Photograph showing karyogram with 47, XX, +8

Chromosomal aberrations were encountered in 20 number of cases in the present study. Out of this hyperploidy was observed in 6 cases, 4 cases with age up to 10 years and 2 cases having age above 10 years. Aneuploidy was observed in only one case of age up to 10 years.

DISCUSSION

Acute myeloid leukemia is a heterogeneous group of diseases with several discrete syndromes having characteristic clinical, morphological and cytogenetic features. Cytogenetic abnormalities were observed in 54.54% of cases, comparable to large studies from different geographic regions (52-80% abnormalities) as stated by various authors [8,9]. Our findings are nearly similar to Enjeti et al. (2004) who reported 61% of abnormal cases in South East Asia region (Singapore) [10]. In the Swedish population-based data there were no differences between male and female occurrence of any chromosomal aberration

[11], which runs parallel to the present study in which gender based differences was not found.

Enjeti et al. (2004) studied 454 patient aged more than 15 years of Singapore population out of which 275 (61%) showed abnormal cytogenetics [10]. Translocation (15;17) was found exclusively in acute promyelocytic leukemia [12]. He described that t(15;17) and trisomy 8 were the most frequent karyotypic abnormalities seen in 52 cases (11%) and 33 cases (7.3%) respectively. In our study these chromosomal aberrations were noted in North Indian population i.e. t (15; 17) in one case (4.54%) and trisomy 8 in two cases (9.09%).

Children with Down syndrome have a 10 to 20 fold increased risk of developing acute leukemia [13-15].

Mandal et al. (2013) found trisomy 21 in one case aged 1 year 8 month in West Bengal (India) region [16] while we noted this trisomy in 3 cases (13.63%) in North Indian population. This type of trisomy was associated also with trisomy 11 in one case (4.54%). but we did not found t(21;21) which was observed by Bakshi et al. (2003) in a 3 year old female child with Down syndrome [17].

Trisomy of chromosome 8 is frequently reported in myeloid lineage disorder and also detected in lymphoid neoplasms as well as solid tumors suggesting its role in neoplastic progression in general. Gain of chromosome 8 was reported in 2 cases (9.09%) in our study. The prognostic significance of extra 8 chromosome in lymphoid malignancies is not reported widely. Trisomy 8 is likely to be a disease modulating secondary event, with underlying cryptic translocation, deletions or mutations as primary events. The role of trisomy 8 in leukemogenesis may be possibly explained by imprinting effect of certain genes due to uniparental disomy caused by the acquired trisomy 8 [18].

Moorman et al. (2002) found trisomy 8 in 32 patients (5.33%) [19]. This frequency was lower to that seen in Taiwan (10%), Japan (12%), but comparable to that in Europe or North America ranging from 6% to 9% [11, 20-23].

Movafagh et al. (2009) reported numerical chromosomal aberration in chromosome no. 5, 7, 8 in 4 cases (6%) of Iranian population out of 65 patients and 5 cases (8%) of Indian population out of 62 patients, which was more frequent in Indian than Iranian population [24]. We observed monosomy of chromosome 8 in one case (4.54%) and trisomy of 8 in two cases (9.09%).

The vast majority of autosomal trisomies results from errors during maternal meiosis. Non-disjunction may occur either during meiosis or mitosis. Postzygotic mitosis errors account for some 5% of nonmosasic autosomal trisomies. Trisomy 11 was reported as an isolated abnormality by Alseraye et al. (2011) in 14 patients of USA [25] and have poor prognosis, but we found that trisomy 11 was associated with trisomy 21 in one case (4.54%).

Ayesh et al. (2012) studied 35 Jordanian Arab patients of aged 16 to 73 years, male to female ratio was 65% males: 35% females out of which chromosomal analysis was possible in 31 cases. Out of these, 20 patients had abnormal karyogram (65%) [26]. He reported that trisomy 8 was the most common to occur in 4 cases (13%), which was also found in the our study in 2 cases (9.09%) but he did not observed t(8;21) in any patient which we noted in two cases (9.09%).

There are only a few population-based studies on AML subjects available in the literature [11, 27]. It is possible that ethnic and geographic factors (or both) could explain some of the variations from the previous findings by different authors.

Large epidemiological studies involving different geographic regions of the world would enable the true nature of environmental and genetic interplay in AML to be completely unfolded.

The present study will be extended by including more number of patients with longer follow- up duration. There are some limitations of present study i.e. chromosomal studies in AML exhibits poor morphology. Chromosomes tend to spread poorly, and appear blurred and fuzzy with indistinct margins, making banding studies challenging. Secondly, minor structural anomalies could not be detected by conventional cytogenetics and further advance techniques like FISH, spectral karyotyping etc. are required to confirm the findings.

CONCLUSION

In this study we observed the chromosomal aberrations in different age groups and sex and also frequency of various chromosomal numerical anomalies in acute myeloid leukemia in North Indian population. Numerical chromosomal abnormality in this study was 31.81% which was different from other population described in previous studies. Trisomy 21 was found to be most prevalent in Uttar Pradesh region as compared to Trisomy 8 and Trisomy 11

associated with Trisomy 21. Trisomy 21 has intermediate risk for developing AML, Trisomy 8 is likely to be a disease modulating secondary event and trisomy 11 has poor prognostic value in AML patients. Hence the cytogenetic analysis of AML patients plays a great role in prognosis and treatment.

REFERENCES

1. Wildonger J and Mann RS. The t (8; 21) translocation converts AML 1 into a constitutive transcriptional repressor. Development. 2005; 132: 2263-72.
2. Burmeister T and Thiel E Molecular genetics in acute and chronic leukemias. J. Cancer Res Clin Oncol. 2001; 127: 80-90.
3. Bacher U, Kern W, Schnittger S et al. Further correlations of morphology according to FAB and WHO classification to cytogenetics in de novo acute myeloid leukemia: a study on 2,235 patients. Ann Hematol. 2005; 84 (12):785-91.
4. Wheatley K, Burnett A, Goldstone A, Gray R, Hann I, Harrison C, Rees J, Stevens R and Walker H. A simple, robust, validated and highly predictive index for the determination of risk-directed therapy in acute myeloid leukemia derived from the MRC AML 10 trial. United Kingdom Medical Research Council's Adult and Childhood Leukemia Working Parties. Br J Haematol. 1999; 107(1): 69–79.
5. Neben K, Jauch A, Hielscher T et al. Progression in smoldering myeloma is independently determined by the chromosomal abnormalities del (17p), t(4;14), gain 1q, hyperdiploidy, and tumor load. Journal of Clinical Oncology. 2013; 31:4325–4332.
6. Caligiuri MA, Schichman SA, Strout MP, Mrózek K, Baer, MR, Frankel SR et al. Molecular rearrangement of the ALL-1 gene in acute myeloid leukemia without cytogenetic evidence of 11q23 chromosomal translocations. Cancer Res.1994; 54:370–3.
7. Mrozek K, Marcucci G, Paschka P, Whitman SP and Bloomfield CD. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood. 2007; 109:431–48.
8. Heim S, Mitelman F (eds). Cancer Cytogenetics. Wiley-Liss: New York. 1995; p.72–73.
9. Ten SK, Khan MK, Khalid H, Lin HP, Ng SC, Cheong SK, Yasmin A and Khuzaiah RR. Abnormalities of chromosome 11Q in three cases of acute myeloid leukemia. Singapore Med J. 1992; 33:164- 166.
10. Enjeti AK, Tien SL, Sivaswaren CR. Cytogenetic abnormalities in de novo acute myeloid leukemia in adults: relation to morphology, age, sex and ethnicity – a single center study from Singapore. The Hematology Journal. 2004; 5: 419–425.
11. Mauritzson N, Johansson B, Albin M, Billstro¨m R, Ahlgren T, Mikoczy Z. et al. A single-center population based consecutive series of 1500 cytogenetically investigated adult hematological malignancies: karyotypic features in relation to morphology, age and gender. Eur J Haematol.1999; 62:95–102.
12. Park TS, Choi JR, Yoon SH et al. Acute promyelocytic leukemia replacing as secondary acute myelogeneous leukemia with translocation t(3;21) (q26;q22) and RUNX1- MDS1-EVIY fusion transcript. Cancer Genet Cytogenet. 2008; 187: 61-73.
13. Fong CT and Brodeur GM. Down's syndrome and leukemia: epidemiology, genetics, cytogenetics and mechanisms of leukemogenesis. Cancer Genet Cytogenet.1987; 28:55-76.
14. Robison LL. Down syndrome and leukemia. Leukemia. 1992;6 (1):5-7.
15. Robison LL, Nesbit ME Jr, Sather HN et al. Down syndrome and acute leukemia in children: a 10-year retrospective survey from Children Cancer Study Group. J Pediatr. 1984; 105:235-242.
16. Mandal A, Mathew S, Saha D and Viswanathan C. Establishment, characterization, and differentiation of a karyotypically normal human embryonic stem cell line from a trisomy-affected embryo. In Vitro Cellular & Developmental Biology-Animal. 2013; 49(1): 15–26.
17. Bakshi C, Amare (Kadam) P, Abhyankar D, Baisane C, Banavali S and Advani S. Chromosomal rearrangement in Down syndrome with acute myeloid leukemia. The Indian Journal of Pediatrics. 2003; 70(9):755-758.
18. Tuna M, Knutila, S and Mills G. Uniparental disomy in cancer. Trends Mol Med. 2009; 15:120 - 8.

19. Moorman AV, Roman E, Cartwright RA, and Morgan GJ. Leukemia Research Fund Centre for Clinical Epidemiology. British Journal of Cancer. 2002; 86: 60 – 62.

20. Johansson B, Mertens F and Mitelman F. Geographic heterogeneity of neoplasia-associated chromosome aberrations. Genes Chromosomes Cancer. 1991; 3 (1): 1–7.

21. Nakase K, Bradstock K, Sartor M, Gottlieb D, Byth K, Kita K et al. Geographic heterogeneity of cellular characteristics of acute myeloid leukemia: a comparative study of Australian and Japanese adult cases. Leukemia. 2000; 14: 163–168

22. Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood. 2000; 96: 4075–4083.

23. Tien HF, Wang CH, Lin MT, Lee FY, Liu MC, Chuang SM et al. Correlation of cytogenetic results with immunophenotype, genotype, clinical features and ras mutation in acute myeloid leukemia. A study of 235 Chinese patients in Taiwan. Cancer Genet Cytogenet.1995; 84:60–68.

24. Movafagh A, Hajifathal, A, Isfahani F, Attarian H, Ghadiani M, Rezvani H, Heidari MH, Miri R and Haji Seyed Javadi M. Geographic Heterogeneity of Cytogenetic Characteristics of Acute Myeloid Leukemia in the early detection: A Comparative Study of Iranian and Indian adult Patients. Iranian Journal of Cancer Prevention. 2009; 2(2): 85-89.

25. Faisal M, Alseraye FM, ZhuangZuo Z and Ramos CB. Trisomy 11 as an isolated abnormality in acute myeloid leukemia is associated with unfavorable prognosis but not with an NPM1 or KIT mutation. Int J Clin Exp Pathol. 2011; 4(4):371-377.

26. Ayesh MH (Haj Yousef). Cytogenetic and morphological analysis of de novo acute myeloid leukemia in adults: a single center study. BJMG. 2012; 15/1:5-10 10.2478/v10034-012-0001.

27. Preiss BS, Kerndrup GB, Schmidt KG, Sorensen AG, Clausen NA, Gadeberg OV et al. On behalf of the AML Study Group Region of Southern Denmark. Cytogenetic findings in adult de novo acute myeloid leukemia. A population-based study of 303/337 patients. Br J Haematol. 2003; 123:219–234.

Original Article

A STUDY OF THE ORBITAL INDEX OF HUMAN DRY SKULLS IN POPULATION OF UTTAR PRADESH

Reena Gupta*, Vasundhara Kulshreshtha*, Anshu Gupta*, Shikky Garg* & Anjali Gupta*
*Department of Anatomy, S.N. Medical College, Agra, UP, India

ABSTRACT

Introduction: The aim of the study was to investigate the orbital anthropometric variation in the normal population. Orbital Index is one of the most important anthropometric parameter. Index varies with race, regions within the same race & period in evaluation. Anthropometric study of orbit is of immense importance on account of its various implications in medical science ranging from ophthalmic surgery, maxilla- facial surgery and neuro-surgery. It is also helpful in plastic surgery (reconstructive surgery) and is used to treat congenital or post traumatic facial disfigurements successfully.

Material and Methods: The study was conducted on 50 dry skulls i.e 100 orbits (both right & left orbits of skull were measured) in Department of Anatomy, S.N Medical College, Agra. The measurement was taken directly using digital vernier caliper. Orbital Index was calculated by measuring orbital height and orbital width in order to evaluate the orbital size & shape.

Results: In the present study, mean orbital height of left orbit was 35.32±6.41 and right orbit was 35.21±6.25 mm, mean orbital width of left orbit was 41.57±6.93 mm and right orbit was 41.73±7.2 mm. Mean orbital index of left orbit was calculated as 82.71±12.10 and for right orbit 82.92±13.02.

Conclusion: The findings of our study suggest that the studied population belongs to microseme category.

Key words: Orbital index, dry skull, digital vernier caliper.

INTRODUCTION

Study of bony orbit is of immense importance on account of its various implications in anthropological and medical science ranging from ophthalmic surgery to plastic surgery (reconstructive surgery) of face. In recent years, traumatology has gained very much clinical and surgical attention especially related to face and orbits. It is a highly important surgico- anatomical landmark for ENT surgeons and Neuro surgeons.

Morphometry is a fast & efficient method for the evaluation of morphological characteristics such as ethnicity, gender, age, genetic factors, dietary habits & regional variation which can alter the shape & size of bone structure. These aspects are significantly important in determining the anthropometic changes between different population and genders [1].

The orbital index which determines the shape of face differs in different population groups. This means that the orbits with larger width than height will have smaller orbital indices. Those with larger orbital index will have narrow face [2].

MATERIAL AND METHODS

This anthropometric study employs the use of direct measurement on dry skulls as it will present a different and more natural prospective in assessing the orbital cavities [3].

The study was conducted on 100 orbits (50 skulls) in Department of Anatomy, S.N Medical College Agra. Fifty human dry skulls i.e. 100 orbits were measured

Address for Correspondence:
Dr. Reena Gupta, Flat No. 506, Kaveri Green Apartment, Kailash Vihar, Bye-Pass Khandari Raod, Agra-282005
Mob: 9837342698 | Email: drreenagupta506@yahoo.com

using digital vernier caliper. Direct measurements of both the right and left orbits were taken with the help of digital vernier caliper.

MEASUREMENTS

In each skull following measurements were taken in both orbits:

1. **Orbital Height (OH)-** Maximum height from the superior to the inferior orbital margin perpendicular to the horizontal axis of the orbit [4] as shown in Fig. 1.
2. **Orbital Width (OW)-**Width was measured from the point of intersection of the anterior lacrimal crest with the frontolacrimal suture (Maxillofrontale) to the most lateral point of the lateral wall of the orbit [4] shown in Fig. 2.
3. **Orbital Index –** Orital index was calculated by the following formula [5]:

$$\frac{Orbital\ height}{Orbital\ width} \times 100$$

Above measurements were compared with studies of different authors as anthropometric measurements are different in different population and geographical areas. So this study will provide data for North Indian adult population.

Fig. 1: Photograph showing measurement of Orbital height of skull

Fig. 2: Photograph showing measurement of Orbital width of skull

OBSERVATIONS

The mean orbital height was found to be 35.21±6.52 and 35.32±6.41 whereas the mean orbital width was 41.73±7.2 and 41.57±6.93 on the right and left side respectively (Table 1).

Table 1: Showing Orbital Height and Orbital Width in 100 Orbits of North Indian Population

	No. of Orbit	Side	Min. (mm)	Max. (mm)	Mean±SD
Orbital height	50	Left	28.91	41.72	35.32±6.41
	50	Right	28.69	41.73	35.21±6.52
Orbital width	50	Left	34.64	48.49	41.57±6.93
	50	Right	34.53	48.93	41.73±7.2

The mean orbital index was 82.92±13.02 and 82.71±12.10 on right and left side respectively (Table 2). Based on this the orbital category of the North Indians is microseme.

Table 2: Showing Orbital Index in 100 Orbits of North Indian Population

	No. of Orbit	Side	Orbital index (Mean±SD)
Orbital Index	50	Left	82.71±12.10
	50	Right	82.92±13.02

DISCUSSION

The index varies with race, regions and within the same race, periods in evolution. The knowledge of the dimensions of the orbital cavity in relation to the skull craniometery is also important in various aspects such as in interpretation of fossil records, skull classification in forensic medicine, in exploring the trends in evolutionary & ethnic differences [3].

Taking orbital index as standard, three classes of orbits have been described. Megaseme (Large) in which the orbital index is 89 or over. This type is seen in yellow races [6]. Mesoseme (intermediate), in this the orbital index ranges between 89 to 83. This type is seen in white races [7]. Microseme (small) are those in which orbital index is 83 or less. This type is characteristic of black races where the orbital opening is rectangular [6].

The orbital index determining the shape of the face differs in different population group. This means that the orbit with larger width than height will have smaller orbital index, while those with larger orbital indices will have narrow faces. This index varies with race, regions within the same race, and periods in evolution (Table 3). This study in North Indian population correlates with the studies done earlier (Table 4). There was little difference observed between the right and left side of orbital index though not significant (Table 2).

Table 3: Orbital Index in Different Population Group [3]

Country	Region	Orbital Index	Category
Japan	Minatogawa	65.2-66.7	Microseme
	Kanto	79.26-80.33	Microseme
	Kinki	79.26-80.33	Microseme
China	PeKing	80.68	Microseme
	Fushun	83.57	Mesoseme
	Hokien	90.35	Megaseme
Malawian		94.35	Megaseme
Indonesia	Flores	106.63	Megaseme
	Batak	99.26	Megaseme
	Klaten	102.73	Megaseme
India	North India[2]	81.65	Microseme
India (Present study)	Uttar Pradesh	82.71-82.92	Microseme

Table 3 & 4 shows the comparison of orbital index between different population groups and also indicate that the orbital index varies with race, region and within the same race as in China PeKing region orbital index was (80.68) of microseme type, in Fushun region orbital index was (83.57) of mesoseme and in Hokien region orbital index was (90.35) of megaseme type. Therefore mixed category of orbital index was found in different regions of one country. This shows that within the same race the orbital index can vary (Table 3). The study conducted in different countries like Nigeria, Japan and India showed that they belong to same microseme category of orbital index (Table 3 & 4) while countries like Malawian and Indonesia showed that they belong to same megaseme category of orbital index. So. orbital index is helpful for physical anthropologists to know the migration pattern of early civilization.

Different studies done in different regions of India i.e. South India, Central India and North India show microseme category of orbital index (Table 4).

Present study done in North Indian population confirms that the orbital index is of microseme category.

Table 4: Comparison of Previous Studies with Present Study

Author	Region	Year of study	Orbital height	Orbital width	Orbital Index	Category
Kaur et al [2]	North India	2012	32.05±2.0	39.25±2.3	81.65	Microseme
Ebeye & Otikpo [8]	Urhobo (Nigeria)	2013	32.46 mm	41.43 mm	78.15	Microseme
Gossavi et al []	Central India	2014	32.31±2.52	39.46±2.57	81.88	Microseme
Girish et al [9]	South India	2014	34.04 (male) 31.12 (female)	41.89 (male) 39.02 (female)	81.13 (male) 81.32 (female)	Microseme
Sidharth Sankar et al [10]	North India	2015	32.91±2.47 (male) 31.83±2.85 (female)	40.55±3.37 (male) 38.73±3.93 (female)	81.15 (male) 82.16 (female)	Microseme
Present study	North India	2017	35.32±6.41 (left orbit) 35.21±6.52 (right orbit)	41.57±6.93 (left orbit) 41.73±72 (right orbit)	82.71±12.10 (left orbit) 82.92±13.02 (right orbit)	Microseme

CONCLUSION

Data collected in the present investigation could serve as data base for the quantitative description of human orbital morphology during normal growth and development considering race and ethnic related variation. The findings of the present study allow for quantification of the orbital features of North Indian population and provide parameters for preoperative planning and prediction of postoperative outcome.

Orbital morphometry is important to provide useful baseline data for ophthalmological, maxillary surgeries and reconstructive cosmetic surgeries of face. Detail knowledge of anatomy and its variations will help the surgeon to avoid surgical complications.

The findings of our study suggest that the North Indian population belongs to microseme category.

REFERENCES

1. Humphrey dean MC and Stringer CB. Morphological variation in great ape and modern human mandibles. Journal of Anatomy. 1999; 195: 491-513.
2. Kaur J, Yadav S, Sing Z. Orbital dimentions - A direct measurement study using dry skulls. J Acad Indus Res. 2012; I(6):293-5.
3. Kumar A and Nagar M. Morphometry of the orbital region: Beauty is bought by judgement of the eyes. International Journal of Anatomy & Research. 2014; 2(3): 566-70.
4. Gosavi SN, Jadhav SD, Zambre BR. A study of orbital morphometry in Indian dry skulls. Asian Journal of Biomedical & Pharmaceutical Sciences. 2014; 4 (29): 23-25.
5. Patnaik VVG, Sanju B, Singla RK. Anatomy of the bony orbit- Some applied aspect. J Anat Soc India. 2001; 50 (1): 59-67.
6. Mcgraw Hill, dictionary of scientific and technical terms "mesoconch" Mcgraw hill company Inc, answer.com (homepage on the internet); Retrieved from http:/ www.answer.com/topic/mesoconch, 2003.
7. Cassidy PJ. "Megaseme" Webster dictionary. Answer. Com (homepage on internet), Retrieved from http:/www.answer.com/topic/megaseme, 1913.
8. Ebeye OA, Otikpo O. Orbital index in Urhobos of Nigeria IOSR-JMDS. 2013; 8(2): 51-53.
9. Patil GV, Shishirkumar, Thejeswari, Apoorva D, Sharif J, Sheshgiri C, Sushanth NK. Study of Orbital Index in human skull of South Indian origin. International Journal of health sciences and research. 2014; 4(9): 125-128.
10. Maharana SS and Agarwal RK. Variations in the Dimensions of the Orbit in the Adult Human Skull (A Dry Bone Study). Int J Rec Biotech. 2015; 3 (3): 20-22.

Original Article

MULTIPLE CHOICE QUESTIONs (MCQs) – AN ITEM ANALYSIS

A.K. Singh*and Richa Niranjan*
*Department of Anatomy, Government Medical College, Haldwani, Nainital, Uttarakhand

ABSTRACT

Introduction: In various educational streams including health sciences, the students are evaluated through subjective and objective questionnaire. Multiple choice questions are to be evaluated for meaningful contribution to the marks obtained by students. This assessment or item analysis is done to make question bank to find out the low achievers.

Material and methods: This study was conducted on 100 first year MBBS students of GMC, Haldwani (UK) on completion of a part of Anatomy. Fifty multiple choice questions were given for item analysis. Difficulty index, the p value and Discrimination index (DI), of multiple choice questions were assessed.

Results: Difficulty index of 24 (48%) items were in acceptable range (p=30-70%), 20 (40%) were optimum range (50-60%) and 6 (12%) were unacceptable range (<30%). Discrimination index of 12 (24%) items were excellent $\geq$ 0.35, 8 (16%) had good discrimination 0.25-0.34, 26 (52) were acceptable 0.20-0.24 and 4 (8%) items were in unacceptable range (<0.20)

Conclusion: Difficulty index, p value >70% and Discrimination index, D value <0.20 needs modification of item or MCQ. Thus this analysis of questions can be useful for development of better questions in the future. MCQ items having optimum Difficulty Index and excellent Discrimination should be regarded as best framed questions.

Key words: Difficulty index, discrimination index, item analysis, multiple choice questions.

INTRODUCTION

Multiple Choice Questions (MCQs) are widely used for MBBS students in colleges as classroom tests and as entrance test for under-graduate and post-graduate courses. A typical MCQ item consists of a question and a set of options that consist of possible answers to the question with single best correct answer. A student's task is to select the one option that provides the best answer to the question asked and one or more usually three distracters of possible answers [1]. A distinct advantage of using MCQ items on classroom tests is that grading tends to be quick and without subjective bias of evaluator. They could be used to measure the most important educational outcomes - knowledge, understanding, judgment and problem solving. By measuring Difficulty Index (p) and Discrimination Index (DI), an examiner can know if an individual question was too difficult or too easy and whether it discriminated better performing students from less performing students.

So it becomes very important that quality of questions be maintained too. For that one may follow the widely accepted item-writing guidelines, such as putting the central idea of the question into the stem and avoiding the use of negation whenever possible [2]. Another way to examine the quality of MCQ items involves analyzing the responses that examinees make, and this is the approach used in the research presented here.

The aim of present study was to check the quality

Address for Correspondence:
Dr. Richa Niranjan, Associate Professor, Department of Anatomy, Government Medical College Haldwani, Nainital, Uttarakhand, Pin-263139
Mob. 9758512594 | Email: niranjanricha@yahoo.co.in

of MCQ items by analyzing the responses and evaluating the Difficulty Index (p) and Discrimination Index (DI) of questions asked in MCQ examination. To identify properly framed questions and questions those need modifications. To prepare the question bank of properly framed MCQ items and give guidelines for the construction of MCQ tests.

MATERIAL AND METHODS

Hundred (100) students of first year MBBS of Government Medical College, Haldwani, Nainital, Uttarakhand were subjected to MCQ test. In this test, "single best option" type of questions were recommended. There were 50 questions in total, all with 4 options including single best option which was considered as correct response. One mark was awarded for each correct response. There were no negative mark for incorrect response. No response was considered as incorrect response. All the responses of all the students were noted and quality of questions were analyzed on the basis of responses. We measured Difficulty Index (how difficult the question was for all the takers) and Discrimination Index (how well the question discriminated more knowledgeable students and less knowledgeable students). Most authors suggest that the discrimination coefficient should be at least +0.20 [3-5].

Difficulty index or Facility value or p value

Accordingly criteria were defined for acceptable and unacceptable questions as following. Difficulty Index (p) was measured as % of correct response from all the students for a particular question.

p = (No. of correct response/No. of total students) x 100 %

Range of p = 0% to 100%

If p is <30% or >70%, it is regarded as unacceptable and MCQ item needs modification. If p is between 30% to 70%, it is acceptable and if p is between 50% to 60%, it is optimum.

Discrimination Index (DI)

DI measurement, merit was prepared on the basis of overall performance of the whole class. Top 30 and bottom 30 students were identified and DI was calculated.

DI = (No. of correct response in top 30 students – No. of correct response in bottom 30 students)/30

Range of DI = -1 to 1

If DI is < 0.20 %, it is regarded as unacceptable and MCQ item needs modification. If it is 0.20 to 0.24, it is acceptable. If it is 0.25 to 0.34it is regarded as good discrimination and if it is 0.35 or more then excellent discrimination.

Statistical Analysis

The scores of 100 students was entered in order of merit in MS Excel 2010 and simple proportions, mean, standard deviation were calculated. Items were categorized according to their difficulty index (p value) and discrimination index (DI).

OBSERVATION AND RESULTS

No. of questions which were asked was 50 and total number of students incorporated in the study were 100. Difficulty index (DI) and Discrimination index was calculated (Table 1 & 2).

Table 1: Interpretation of Difficulty Index (p)

Range	Interpretation	No. of Questions	%
<30% or >70%	Unacceptable	**06**	**12%**
30%-70%	Acceptable	**24**	**48%**
50%-60%	Optimum	**20**	**40%**

Table 1 interprets that from total number of 50 MCQs, 24 (48%) were acceptable as correct answer were given by all students, 20 (40%) questions were optimum as right answer was given by some students and 6 (12%) questions were unacceptable as they were not attempted by students.

Table 2: Interpretation of Discrimination Index (DI)

Range	Interpretation	No. of Questions	%
<0.20	Unacceptable	**04**	**8%**
0.20-0.24	Acceptable	**26**	**52%**
0.25-0.34	Good discrimination	**08**	**16%**
≥0.35	Excellent discrimination	**12**	**24%**

Table 2 finds out performance of students in class through the discrimination index. Out of total 50 MCQs, 12 (24%) questions had excellent discrimination, 8 (16%) had good discrimination, 26 (52%) are acceptable discrimination and only 4 (8%) questions were unacceptable.

DISCUSSION

As seen in Table 1, there were 06 questions with Difficulty Index (p) <30% or >70%. So they required modification before they can be considered as standard questions. Twenty four questions were within acceptable range of Difficulty Index (p) and out of those 24, twenty questions were of Difficulty Index (p) between 50% and 60%. So they can be considered as optimum as far as difficulty is concerned.

As seen in Table 2, Discrimination Index (DI) of 4 out of 30 questions was below 0.20 and hence unacceptable. DI of rest of the questions were >0.20 and so acceptable with 08 questions were categorized as having Good Discrimination (DI= 0.25 to 0.34). 12 questions were categorized as having Excellent Discrimination (DI= 0.35 or more).

A thoughtfully written MCQ items can serve to assess higher-level cognitive processes, although creating such items does require more skill than writing memory-based items [6,7].

According to Downing (2003), item writing is both art and science [8]. There are scientifically sound principles of item writing but creation of effective multiple choices item requires the skillful application of these principles to the content to be tested. The content of an item should be specific, important and independent in simple language. Tricky, opinion based item and complex multiple choice format should be avoided. Correct use of grammar, punctuation, capitalization and spellings. Minimal amount of reading in each item is required. In options of an item only one answer should be correct. Option should in logical or numerical order. Choice should not overlap in meaning and length of choices should be equal. None of the above and all of the above choices must be avoided. All distracters should be plausible and typical student's errors. The general directions for multiple choice items usually require selecting the best answer from those presented, although some test requires selecting the single correct answer from those given. The best answer format is most common because it is most defensible.

Bacon (2003) compared multiple choice test and short answer test in terms of time of completion, reliability and validity [9]. The MCQs were found to yield equivalent reliability and validity in a shorter amount of test taking time and no gender effect was found. One criticism is that the format of MCQ item let students guess even when they have no substantive knowledge of the topic under consideration [10]. Use of MCQ as testing method in MBBS curriculum is increasing.

CONCLUSION

It can be concluded from the present research that Difficulty Index (p) and Discrimination Index (DI) are very nice tool for the assessment of the quality of an MCQ item. An MCQ item should be considered unacceptable and modified to get difficulty level and discrimination power within acceptable range before it can be included in a standard MCQ bank.

All the postgraduate students and faculty members including Head of the institute were delighted to know the Difficulty Index (p) and Discrimination Index (DI) for the assessment of the quality of an MCQ item. According to faculty members, it is a very nice tool for making a standard MCQ bank and measuring the educational outcomes i.e. knowledge, understanding, judgment and problem solving.

REFERENCES

1. DiBattista D & Kurzawa L. Examination of the quality of Multiple-choice items on classroom tests. The Canadian Journal for the Scholarship of Teaching and Learning. 2011; 2 (2): Article 4.
2. Haladyna TM, Downing SM, Rodriguez MC. A review of multiple-choice item writing guidelines for classroom assessment. Applied Measurement in Education. 2002; 15 (3):309-344.
3. Ding L & Beichner R. Approaches to data analysis of multiple-choice questions. Physical Review Special Topics–Physics Education Research. 2009; 5, 020103.
4. Su W, Osisek PJ, Montgomery C, Pellar S. Designing multiple choice test items at higher cognitive levels. Nurse Educator. 2009; 34 (5): 223-227.

5. Thorndike RM. Measurement and evaluation in psychology and education. Upper Saddle River, NJ: Pearson, 2005.
6. Buckles S & Siegfried JJ. Using in-depth multiple-choice questions to evaluate in depth learning of economics. Journal of Economic Education. 2006; 37 (1): 48-57.
7. Palmer EJ & Devitt PG. Assessment of higher order cognitive skills in undergraduate education: modified essay or multiple choice questions? BMC Medical Education. 2007; 7: 49.
8. Downing SM. Guessing on selected-response examinations. Medical Education. 2003; 37: 670-671.
9. Bacon DR. Assessing learning outcomes: A comparison of multiple-choice and short answer questions in a marketing context. Journal of Marketing Education. 2003; 25:31-36.
10. Biggs J & Tang C. Teaching for quality learning at University. Buckingham, UK: Society for Research into Higher Education and Open University Press. McGraw-Hill Education, 3rd ed., 2007.

Original Article

IMPACT OF BODY PAINTING IN TEACHING SURFACE ANATOMY

Sudipa Biswas*, Suranjali Sharma*, Soumya Chakraborty*, Ankana Saha*
*Department of Anatomy, ESI PGI-MSR and ESIC Medical College, Joka, Kolkata, (West Bengal, India)

ABSTRACT

Introduction: Surface anatomy is widely used as a tool for teaching anatomy. Students participate in this teaching method and try to reproduce the same as shown by the faculty in examination. It has been noted that during assessment, the performance of the student is quite poor. In most of the cases students fail to show the defined structure over the surface of a living subject even when there is enough theoretical knowledge. It may be due to the fact, students hardly enjoy this method of teaching and fail to perform accordingly during assessment, or due to unavailability of adequate volunteers during teaching.

Aim of the study: Nowadays a lot of improvement has been observed in the fields of teaching methodology of anatomy like, power point presentation in lecture classes, use of videos for easy demonstration of a complex procedure of embryological development, digitalization of histology slide for demonstration, use of virtual microscopy and other technologically advanced programs. Therefore, the purpose of this study was to identify the role of teaching surface anatomy with some modifications like, body painting and impact of body painting in teaching surface anatomy.

Methods: Ninety four (94) undergraduate medical students participated in the study. Their responses were tabulated in 6 point Likert's scale through both pre and post test questionnaires.

Results: Students were actively involved in the session and found the session interesting. They agreed that it was useful in memorizing the surface features along with learning from each other (peer).

Conclusion: The analysis showed positive impact of body painting as a tool for teaching surface anatomy.

Key words: Surface anatomy, body painting, visual images.

INTRODUCTION

It has been appropriately said that living and surface anatomy forms the bridge between clinical practice and gross anatomy [1]. Live feel of pulsations, body land marks etc. helps in building better clinical /practical ideas as compared to cadaveric anatomy. The living anatomy, defined as the anatomy which is revealed on the living humans, is gaining importance in the modern anatomy education, and it has even been considered to replace the cadaver-based anatomy study [2] in many centres; the dearth of cadaver for dissection is one of the reasons for considering other teaching learning tools in anatomy.

In traditional method of surface anatomy, students put a lot of effort to memorize the surface features and often forget the landmarks during the assessments. Success of any teaching method depends on the acceptance and its ease of reproduction as the need

Address for Correspondence:
Dr. Suranjali Sharma, Assistant Professor, 5A, Block I, Suvenior Enclave, 8/1 Jessore Road, Dum Dum, Kolkata– 700028
Mob: 9836011390 | Email: suranjali.sharma@gmail.com

be. With advancement of medical technology and time constraint, educationists are implementing newer teaching methodologies like body painting, virtual anatomy imaging software, peer examination, living anatomy model, and full body digital X-Ray in combination with palpation of landmarks on peers and cadavers, supplemented with self-directed learning [3,4]. In 2008, American Association of Anatomists, on the basis of their experience and student feedback, strongly advocated the use of body painting as an adjunct to surface anatomy and clinical skills teaching classes [5].

In this study, we used body painting exercises for the students to learn the surface anatomy of a few selected regions. The students were actively engaged during the whole session. The colorful images made by themselves, motivated the students for the session and helped them retaining the knowledge acquired.

The aim and objectives of this study was to identify the awareness among students about the importance of surface anatomy; to assess the interest of the participants for body painting over traditional tool; to identify the responses of doing body painting among students like willingness in participation, memorization of surface features, apprehension regarding the peer-peer examination, retention of knowledge and limitations of body painting and to compare the impact of both the procedures (traditional surface anatomy and body painting).

The willingness involved in this method of teaching as compared to the formal teaching sessions helps in building better teaching learning activities. In this study, we tried to assess whether body painting can be considered as supplement to traditional surface anatomy sessions.

METHODS

Study was done in ESI PGI-MSR and ESIC Medical College, Joka. Kolkata. A written consent from the students and ethical clearance for the project from the institutional ethical committee was obtained.

94 out of 100 first year medical students (Batch 2016-2017) had been asked about their ideas of importance of surface anatomy, using open and close ended structured questionnaire. Also they participated in body painting sessions of a few selected regions like nerves at back of hand (Fig.1), nerves in palm (Fig.2), spaces of hand (Fig.3), synovial sheaths in hand (Fig.4) etc.

Fig. 1: Photograph showing painting of nerves at back of hand

Fig. 2: Photograph showing painting of nerves in palm

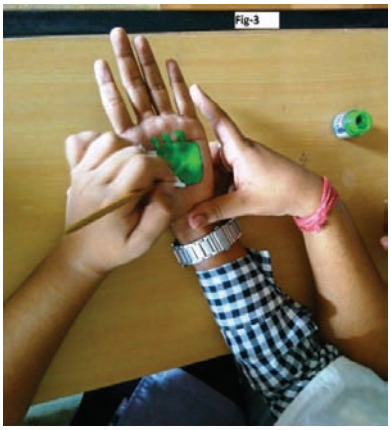

Fig. 3: Photograph showing painting of palmar spaces of hand

Fig. 4: Photograph showing painting of synovial sheaths in hand

One group (47 students) was allotted 25 minutes to paint on another group (47 students) and in the next 25 minutes groups were interchanged. Non toxic water based body paints of red, green and yellow colours and brushes of different sizes were provided for the body painting. Each and every student underwent the sensitivity test for the colours which were used, before the session.

After the session, the students were asked about their experience through the questionnaire.

Their responses were noted with a set of statements by using a six point Likert scale.

RESULTS

94 students (55 male i.e. 58.51% and 39 female i.e. 41.49%) participated in this study and filled up the feedback questionnaire for both the pre and the post tests. Reliability and validity of 6-point scales were assessed using a new model-based approach to fit empirical data. The 6-point Likert scale which reads:

Very strongly Disagree (response 1)

Strongly Disagree (response 2)

Disagree (response 3)

Agree (response 4)

Strongly agree (response 5)

Very strongly agree (response 6)

Questionnaire was as follows:

1. The learning tool is interesting-
2. Actively involved-
3. Easy identification of surface features-
4. Helps in one to one learning-
5. Direct and live feel of surface features-
6. Remembering the points is easy-
7. Procedure can be used everywhere-
8. Body painting can be used as a tool in place of surface anatomy -
9. Give your opinion regarding the procedure-

Table 1: Percentage of students responses based on the pre-test and post-test (cumulative form)

	Pre-Test		Post-Test	
Questions	**Positive Responses**	**Negative Responses**	**Positive Responses**	**Negative Responses**
	Number of students responses (%)	Number of students responses (%)	Number of students responses (%)	Number of students responses (%)
1	86 (91.49%)	8 (8.51%)	94 (100%)	0 (0%)
2	62 (65.96%)	32 (32.04%)	93 (98.94%)	1 (1.06%)
3	55 (58.51%)	39 (41.49%)	94 (100%)	0 (0%)
4	73 (77.66%)	21 (22.34%)	94 (100%)	0 (0%)
5	71 (75.53%)	23 (24.47%)	93 (98.94%)	1 (1.06%)
6	44 (46.81%)	50 (53.19%)	93 (98.94%)	1 (1.06%)
7	54 (57.45%)	40 (42.55%)	80 (85.11%)	14 (14.89%)
8	74 (78.72%)	20 (21.28%)	93 (98.94%)	1 (1.06%)

Table 1 helps to show the number of responses for each Likert level in each question and also distinguishes between pre-test and post-test student responses.

In post-test, 100% of the students agreed that the body painting method of learning was interesting. 98.94% agreed that they actively involved in the body

painting sessions as compared to 65.96% in traditional surface marking classes. 100% as compared to 58.51% agreed that it was easy to list out the surface features after the body painting exercise. 100% students in contrast to 77.66% agreed that painting on each other facilitated a one to one learning. 98.94% agreed that body painting gave them the feel of the landmarks (surface features) and various structures. 98.94% agreed that the remembering of knowledge was better when it was learnt through body painting. 85.11% agreed that post-test procedure can be used everywhere compared to 57.45% agreed in pre-test procedures.

More number of positive responses was in post-test (97.61%) comparative with pre-test (69.02%).

Cronbach's alpha is a measure used to assess the reliability, or internal consistency, of a set of scale or test items [6]. In this current study, the Cronbach's alpha was found 0.753, which is acceptable.

Table 2: Relation between pre-test and post-test in each section

		Average	Most Frequent responses (Mode)	Responses
Q1	Pre Test	4.17	4	Agree
	Post Test	5.52	6	Very Strongly Agree
Q2	Pre Test	3.77	4	Agree
	Post Test	5.38	6	Very Strongly Agree
Q3	Pre Test	3.70	4	Agree
	Post Test	5.14	6	Very Strongly Agree
Q4	Pre Test	3.97	4	Agree
	Post Test	5.21	6	Very Strongly Agree
Q5	Pre Test	3.99	4	Agree
	Post Test	5.27	5	Strongly Agree
Q6	Pre Test	3.49	3	Disagree
	Post Test	5.22	4	Agree
Q7	Pre Test	3.69	4	Agree
	Post Test	4.46	4	Agree
Q8	Pre Test	4.12	4	Agree
	Post Test	5.19	6	Very Strongly Agree

Table 2 indicates the average value of responses in both testing tools (pre-test and post-test). The current table also identified the mode, which measures the most frequent score in study data set. In every scenario, mode value was higher in post-test comparative with pre-test. But post-test tool get better responses compared to the pre-test tool (Fig.5).

So, Table 2 indicates that in every scenario post-test responses were more positive comparative to pre-test.

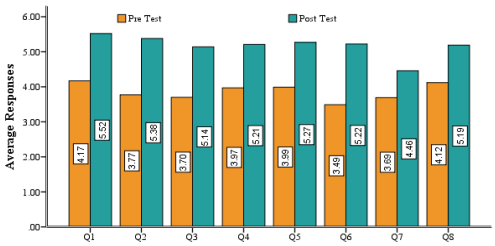

Fig. 5: Bar diagram showing average value of responses in pre-test and post-test

Table 3: Association between pre-test and post-test tool in each questions

		P value	Significance
1	Pre Test	.001	Significant
	Post Test		
2	Pre Test	.001	Significant
	Post Test		
3	Pre Test	.001	Significant
	Post Test		
4	Pre Test	.001	Significant
	Post Test		
5	Pre Test	.001	Significant
	Post Test		
6	Pre Test	.001	Significant
	Post Test		
7	Pre Test	.001	Significant
	Post Test		
8	Pre Test	.001	Significant
	Post Test		

Table 3 indicates the association between two tools. There were highly significant differences found between pre-test and post-test tools (p = .001) using Mann-Whitney non parametric test.

From this study we learned that body painting is active and interesting learning tool, good for memorizing surface features, helpful in retention of knowledge, forming of comfort zone among peers and revision can be done among peers in their out of curriculum activity thus saving already compromised formal curriculum time.

DISCUSSION

In many studies done previously the result of using body painting in surface anatomy classes, showed good results. This study tested the utility of implementing the same technique in our institution in eastern part of India. As such, this tool is not formally used for teaching in medical institutions in India to the best of my knowledge.

Since surface anatomy is one of the important learning skill needed for future clinical orientation, this process of understanding deeper structures needs to be improved according to the modern technology since the beginning of the course.

It has been well studied and concluded in a study that inclusion and implementation of teaching and learning strategies, such as body painting, peer physical examination, medical imaging and virtual anatomy softwares will enhance learning of living and surface anatomy in an integrated and relevant framework [7]. The success of a program depends very much on the interest of the participant. Akker et al. (2002) designed a course in which the student painted the full organ at the site of its projection on the body surface. The course was successful and enjoyed by students [8].

In our study also, 100% students agreed that this was as an interesting procedure. The finding was similar to study done by Nanjundaiah & Chowdapurkar (2012) [9]. Nowadays self-directed learning is being promoted in medical courses. It is well understood now that body painting increases both student motivation and knowledge acquisition though there are some other options like, using life models. In our study, active involvement by students as compared to traditional method was 98.94% which is again similar to the previous study [9].

On the basis of experience and student feedback, McMenamin (2008), suggested that the use of body painting as an adjunct to surface anatomy and clinical skills teaching classes. It may have the added bonus of helping break down the apprehension regarding the peer-peer examination [5]. They even learned from their peer partners. In our study same has been reflected (100% agreed in one to one learning). There were better responses for direct and live feel of surface features in body painting (98.94%) as compared to traditional surface anatomy classes (75.5%) which made the post-test tool more successful. Students even accepted that the body painting procedure increased their retention power of knowledge more (98.9% vs. 46.8%) as compared to traditional method (Table 1).

It is well said that, a successful learning of anatomy requires a balance between the memorization with understanding and the visualization [10]. In the post-test tool, colorful images may help in remembering the points in future. Use of colours encouraged 100% students of our study in easy identification and memorization of surface features (Table 1).

There are a lot of positive outputs with body painting like; relatively inexpensive, associated motivation and enthusiasm among students [11]. In this study we also felt the same vibe which reflected in their open ended post test questionnaire.

There may be a few drawbacks like, female students from ethnic minority groups may be reluctant to volunteer as seen in study by Aggarwal et al. (2006) [12]. Single-sex classes may improve volunteer rate. Though in the present study, this problem had not surfaced and all the students participated voluntarily and actively irrespective of sex and ethnicity.

It should be accepted that the scope of including all the parts of body for body painting is limited. According to Table 1, 14.89% students in post test disagreed with the fact that body painting may be used all over the body surface. Also we felt that the process needs special preparations and time.

In a study done in 2011 it has been concluded that the use of colour had no impact on short-term or long-term retention of knowledge, despite previously collected qualitative data that colour helped memorization [13]. In present study, finally 98.94% students agreed that body painting may be used as a tool in place of surface anatomy.

CONCLUSION

There is always a need for understanding the surface anatomy as it gives students idea about texture, depth and boundaries of underlying structures like bones, tendons, vessels, viscera and different organs etc. by touching and feeling. These ideas help students to understand the importance of clinical assessment during medical practice in future. This skill helps the surgeons to plan out the incisions, like in minimally invasive surgeries (arthroscopy, laparoscopy etc). It helps the physician in diagnosing tenderness of deep seated organs, anesthetists to assess position of trachea during intubation and central or peripheral vascular access etc. Today with modern technologies like USG, MRI, and CT scans, though doctors can readily diagnose the underlying pathology but still we cannot deny the importance of the bedside clinical methods. The knowledge is very useful in acute trauma and emergency procedures, as advocated in ATLS (advanced trauma life support) protocol for procedures like emergency needle cricothyroidotomy, needle insertion for tension pneumothorax, chest tube insertion etc. Therefore even in modern era the usefulness of using surface anatomy is immense.

In Table 2, the relation between Pre and Post-test showed how in every scenario Post-test responses were more positive comparative to Pre-test. Fig. 5 showed graphical representation of average responses of both the tests in every scenario. Hereby, we can come to the conclusion that using Post-test tool there were positive responses comparative to Pre-test tool.

The body painting therefore, may be supplemented properly with traditional anatomy, clinical skills, drawing and labelling of the body parts.

This study re-establishes the role of surface anatomy which may be supplemented with body painting. The results were showing positive feedback from the students for the body painting (Table 3). There were highly significant differences found between Pre and Post-test tools (P=.001).

The students can use this tool (body painting) anytime and also can practice it anywhere. The students opined that not all the regions in our body can be painted and this is a time consuming process. Therefore, restructuring the sessions of body painting may help the students in future.

Body painting may be considered as a method of teaching surface anatomy in addition to the traditional surface anatomy teaching sessions.

Conflict of interest: None

Acknowledgements: I like to acknowledge all the teaching and non-teaching staff of Department of Anatomy, ESIC Medical College, Joka, Kolkata, for their immense support during the whole study.

REFERENCES

1. Teichgräber UK, Meyer JM, Berens von Rautenfeld D. Teaching applied anatomy to senior medical students with an emphasis on surgery and radiology. Surg Radiol Anat. 1996; 18(2):141–142.
2. Ganguly PK, Chanz LK. The living anatomy in the 21st century: how far can we go? South East Asian Journal of Medical Education. 2008; 2 (2): 52-57.
3. Finn GM. Twelve tips for running a successful body painting teaching session. Med Teach. 2010; 32(11):887-90.
4. Azer SA. The place of surface anatomy in medical literature and undergraduate anatomy text books. Anat Sci Educ. 2013; 6(6):415-32.
5. McMenamin PG. Body-painting as a tool in clinical anatomy teaching. Anatomical Sciences Education. 2008; 1(4): 139-44.
6. Lee J Cronbach. My Current Thoughts on Coefficient Alpha and Successor Procedures. Educational and Psychological Measurement, Edited by Shavelson RJ. 2004 Sage Publications. 2004; 64 (3): 391–418.
7. Asad MR, Nasir N. Role of Living and Surface Anatomy in Current Trends of Medical Education. IJARIIE-ISSN (O)-2395-4396. 2015; 1(2).
8. Op Den Akker JW, Bohnen A, Oudegeest WJ, Hillen B. Giving color to a new curriculum: Body paint as a tool in medical education. Clin Anat. 2002; 15(5): 356-62.
9. Nanjundaiah K, Chowdapurkar S. Body Painting: A Tool Which Can Be Used to Teach Surface Anatomy. Journal of Clinical and Diagnostic Research. 2012; 6(8): 1405-1408.
10. Pandey P, Zimitat C. The medical students' learning of the human anatomy: memorisation, understanding and visualization. Medical education. 2007; 41(1):7-14.
11. Cody J. Painting anatomy on anatomy. J Biocommun. 1995; 22:14–17.

12. Aggarwal R, Brough H, Ellis H. Medical student participation in surface anatomy classes. Clin Anat. 2006; 19(7):627-31.

13. Finn GM, White PM, Abdelbagi I. The impact of color and role on retention of knowledge a body-painting study within undergraduate medicine. Anat Sci Educ. 2011; 4 (6): 311–317.

Original Article

HISTOLOGICAL PROFILE OF KIDNEY OF ALBINO RATS ON ORAL ADMINISTRATION OF SODIUM BENZOATE

Rashmi Kumari*, Archana Sharma*, G.L. Nigam*, Ketu Chauhan*, Arvind Yadav*, Anuj Malik*
*Department of Anatomy, LLRM Medical College, Meerut

ABSTRACT

Introduction: Sodium benzoate is a food preservative that is present in many food stuff as antimicrobials. The damaging effects of sodium benzoate on the kidney of albino rats were studied.

Material & Methods: Forty albino rats randomly divided into four groups. Group I served as control which received distilled water. Group II, III & IV received 400mg/kg, 800mg/kg and 1200mg/kg of sodium benzoate respectively via intragastric route. After 30 days, the animals were sacrificed and kidney changes were observed by light microscope.

Observations: We observed a general architectural derangement in the form of glomerular tufting, decreased Bowman's space, dilatation of tubules, thickened basement membrane, cloudy swelling in tubules and whole of the kidney was found to be infiltrated with inflammatory cells.

Keywords: Sodium benzoate, food preservatives, histological changes, kidney.

INTRODUCTION

The kidney problems are increasing day by day and now proved that a number of chemical compounds which are present in our food is responsible for it. One of the food preservatives sodium benzoate is also responsible for that. Sodium benzoate is also present naturally in many food stuffs and in plant extract but they are used as preservative for different food categories, including dairy based deserts, margarine and emulsion. These chemical compounds may be antioxidants and antimicrobials. The sodium benzoate is classified as class II preservatives with the allowed maximum permissible concentration of 1000ppm [1].

They inhibit the growth of mould, yeast and some bacteria by lowering the intracellular pH to less than '5'. This decreases the anaerobic fermentation of glucose by almost 95%. So, benzoates are used as antimicrobial agent (at level 0.05-0.1%) in acidic food & beverages [2].

Sodium benzoate ($C_7H_5O_2$ Na-Sodium salt of benzoic acid), having molecular weight of 144.11 has a melting point about 300^0C. It is very soluble in water (550-630 g/litre at 200^0C) and is hygroscopic at a relative humidity about 50%. Its pH is about 7.5 at a concentration of 10 g/litre water. It is soluble in ethanol, methanol, and ethylene glycol [3].

The present study has been conducted to elucidate histological changes in the kidney of albino rats on oral administration of sodium benzoate.

MATERIAL AND METHODS

The present study was conducted in Department of Anatomy, LLRM Medical College, Meerut, UP after obtaining approval from the Institutional Ethical Committee for evaluating histological effects of sodium benzoate administration orally on kidney of albino rats.

Address for Correspondence:
Dr. Rashmi Kumari, Department of Anatomy, LLRM Medical College, Meerut
Mob: 9634147912 | Email: rashmi4kumari2000@gmail.com

Forty albino rats of both sexes, weighing about 150 ± 50g each were used in this study. They were divided into four groups of 10 rats in each group. Group I was considered as control group and received distilled water only, group II, III and IV were given 400 mg/kg, 800 mg/kg and 1200 mg/kg body weight sodium benzoate in distilled water, respectively for 30 days.

The animals were sacrificed after 30 days of experiment. Kidney tissue was fixed in 10% buffered formaline and stained with haematoxyline and eosin.

OBSERVATIONS

Dose dependent histopathological changes were observed in the kidney of most of the rats after 30 days.

A general architectural derangement in the form of glomerular tufting, proximal tubular dilatation, loss of brush border and decreased Bowman's space were found. Cloudy swelling in tubules was also found. Whole of the kidney was found to be infiltrated with inflammatory cells. Increased cellularity of glomerulus, decreased Bowman's space, thickening of basement membrane was also noticed (Fig.1-8). Number of albino rats showing histopathological changes is enumerated in Table 1.

Table 1: Histopathological changes in kidney of albino rats after 30 days

Features	Groups			
	Control Group	Experimental Group		
	I	II	III	IV
Derangement of Glomerular architecture	-	6	7	9
Obliterated Bowman's space	-	-	7	7
Increased cellularity in some glomeruli	-	6	7	9
Inflammatory cell infiltration	-	7	9	10
Cloudy swelling in tubule	-	6	8	9
Blood vessel dilatation	-	7	7	9

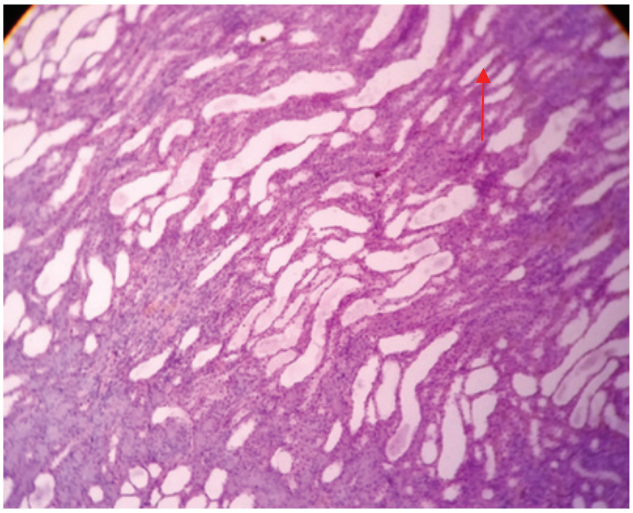

Fig. 1: Plate of kidney section of medulla of white albino rat (control) at 100 X showing normal tubules (red arrow) after 30 days

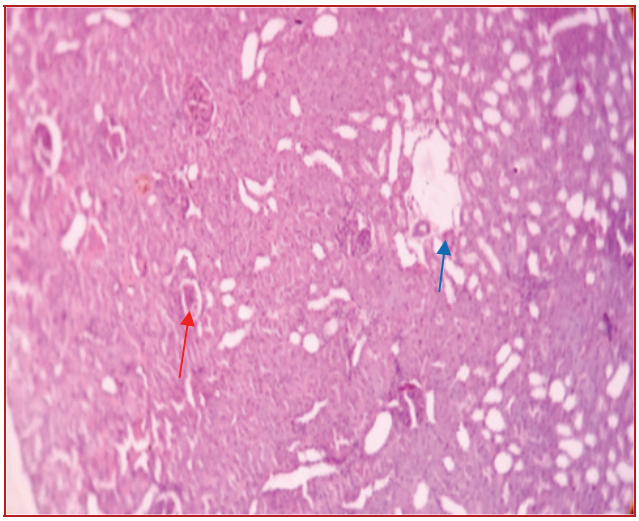

Fig. 2: Plate of kidney section of cortex of white albino rat (control) at 100 X showing normal glomeruli (red arrow) and blood vessel (blue arrow) after 30 days

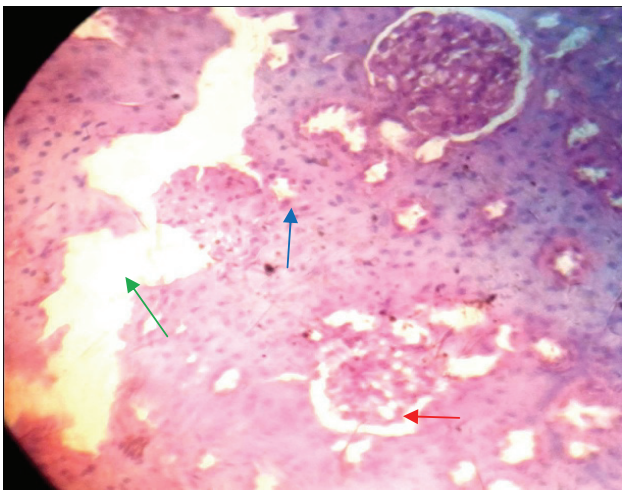

Fig. 3: Plate of kidney section of cortex of white albino rat in experimental group I given 400 mg/kg body weight of sodium benzoate for 30 days at 400 X showing derangement of glomerular architecture (red arrow), inflammatory cell infiltration (blue arrow), space devoid of renal tissue (green arrow)

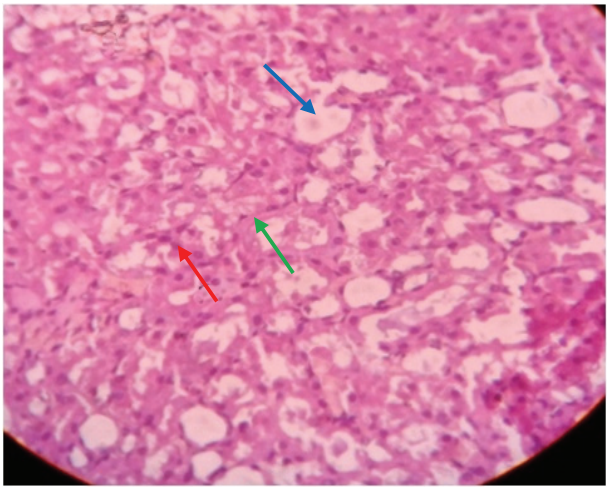

Fig. 5: Plate of kidney section of medulla of white albino rat in experimental group II given 800 mg/kg body weight of sodium benzoate for 30 days at 400 X showing inflammatory cell infiltration and loss of tubular architecture (red arrow), cloudy swelling in tubule (green arrow), dilatation of tubule (blue arrow)

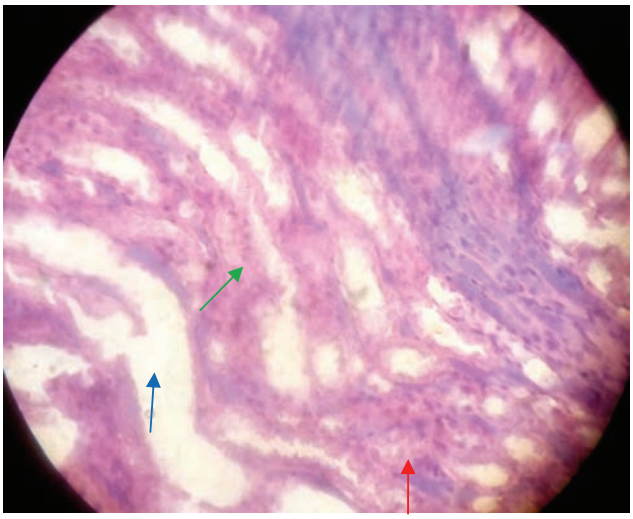

Fig. 4: Plate of kidney section of medulla of white albino rat in experimental group I given 400 mg/kg body weight of sodium benzoate for 30 days at 400 X showing inflammatory cell infiltration and loss of tubular architecture (red arrow), cloudy swelling in tubule (green arrow), dilatation of tubule (blue arrow)

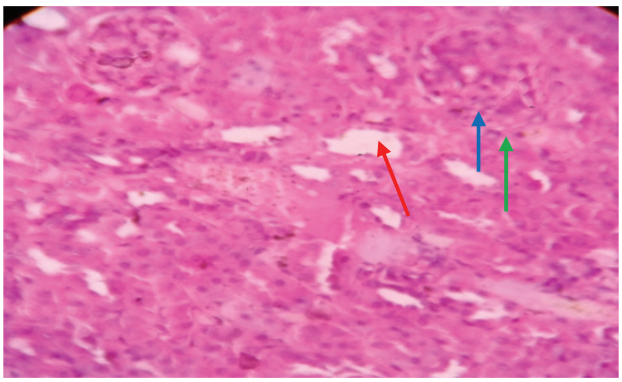

Fig. 6: Plate of kidney section of cortex of white albino rat in experimental group II given 800 mg/kg body weight of sodium benzoate for 30 days at 400 X showing derangement of glomerular architecture (green arrow), obliterated Bowman's space (blue arrow), space devoid of renal tissue (red arrow)

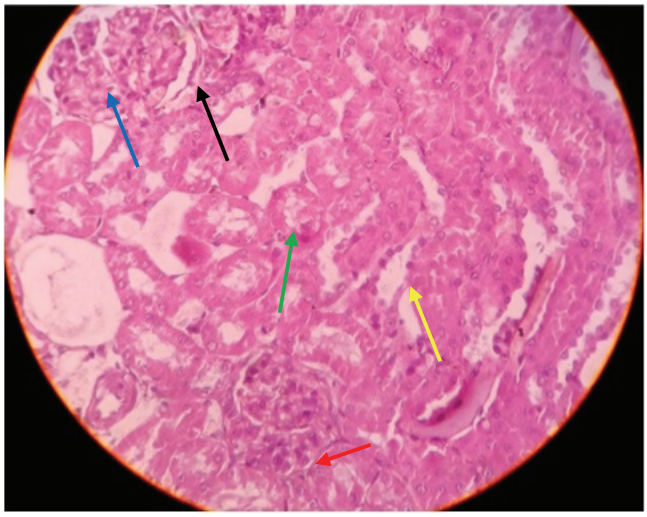

Fig. 7: Plate of kidney section of cortex of white albino rat in experimental group III given 1200 mg/kg body weight of sodium benzoate for 30 days at 400 X showing derangement of glomerular architecture and obliterated Bowman's space (red arrow), thickened basement membrane (black arrow), increased cellularity in some glomeruli and glomerular tufting (blue arrow), inflammatory cell infiltration and loss of tubular architecture (yellow arrow), cloudy swelling in tubule (green arrow)

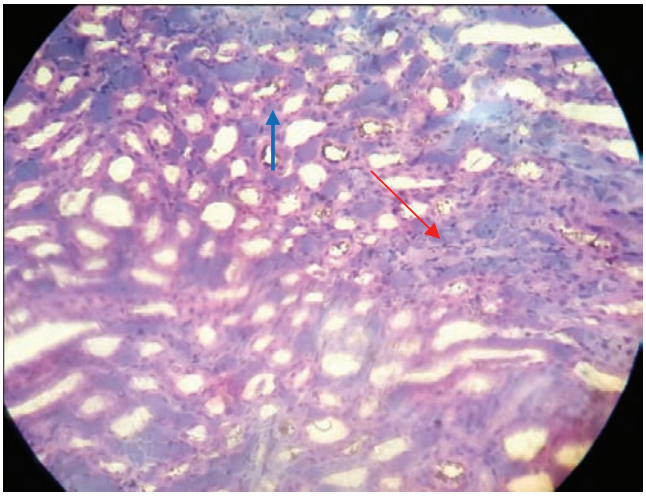

Fig. 8: Plate of kidney section of medulla of white albino rat in experimental group III given 1200 mg/kg body weight of sodium benzoate for 30 days at 400 X showing inflammatory cell infiltration and loss of tubular architecture (red arrow), narrowing of tubule (blue arrow)

DISCUSSION

The kidney of control rats showed normal histological structure of the renal corpuscles and renal tubules on light microscopy.

In our study, derangement of glomerular architecture, increased cellularity in some glomeruli etc. was found with almost all doses i.e. 400mg/kg, 800mg/kg and 1200mg/kg body weight. The severity of changes increased with higher doses. The findings of our study runs parallel with the findings of other observers [2,4-7]. Algadir et al. (2009) [2] found lobulated tuft and desquamated cells. Most glomeruli appeared hypercellular with 1250mg/kg dose. According to them, the cause of this derangement is inflammation and damage to kidneys. Chen et al (2014) [7] showed shrinkage of glomeruli, widened urinary spaces and capillary congestion. They attributed these changes to the change in related enzyme activities and changes in membrane permeability. Mohamed (2012) [5] observed few renal corpuscles were completely degenerated and leaving empty spaces.

Another important finding on light microscopic examination revealed marked degenerative lesions in many renal tubules. The tubular cells showed swelling with cloudy swelling in tubules, loss of brush border, small and deeply stained nuclei etc. These findings are also in consensus with findings of Shide and Chandrasekaran (2011) who observed that the tubular cells displayed small and deeply stained nuclei. Patches of leucocytes mainly formed of mononuclear cells were also found between kidney tubules [8].

This is justifiable since the renal tubules are in contact with toxic chemicals during their excretion and elimination [9].

The observed necrosis of the most of the renal tubules and glomeruli may be related to the depletion of ATP, which finally leads to the death of the cells [10].

In our study, we have also found inflammatory cell infiltration in maximum number of rats. This was also observed by Khayyat et al. [4]. They explained that this inflammation may be due to microcirculatory disturbances [4].

CONCLUSION

Food preservatives like sodium benzoate used for prolonging shelf-life of the prepared food, was seen to cause degenerative changes in tissue. Degenerative

changes like cloudy swelling, inflammatory cell infiltration, decreased Bowman's space and increased cellularity of the glomeruli have been observed in kidney of albino rats in this study after administration of sodium benzoate orally for 30 days.

So, the above mentioned degenerative changes are clearly indicative of excretion of the compound mainly through kidney.

REFERENCES

1. Health Canada – Food and Nutrition. List of permitted preservatives (List of permitted food additives) super seeds -2014 -10-23 Date Issued: 2015-02-19.
2. Algadir MIA, Ihaimer MM, Kikhier MKS, Idir QR. Effects of Benzoic acid with citric acid as food additives on the renal function of experimental rats. Asian journal of clinical nutrition 2009; 1(2): 83-87.
3. Maki T, Suzuk Y. Benzoic acid and derivatives. In Ullmann's encyclopedia of industrial chemistry. Vol. A3. Weinheim, VCH Verlagsgesellschaft mbH, pp. 555-568.
4. Khayyat L, Essawy A, Sorour J, Al Rawi M. Impact of some energy drinks on the structure and function of the kidney in Wistar Albino rats. Life science journal 2014; 11(10):1131-1138.
5. Mohamed SM. Histological and histochemical changes on the liver and kidney of adult male albino rat after the administration of sodium benzoate. 2012; 41(1):11-39.
6. Aktac T & Bakar E. Effects of sodium benzoate and citric acid on serum, liver and kidney tissue total sialic acid levels. An ultrastructural study. Journal of Applied Biological Sciences 2014; 8(2): 09-15.
7. Xg Chen, QxLv, Ym iu, Wdeng. Effects of the food additives, citric acid on kidney cells of mice. Informa Health care 2014; 90(1):38-44.
8. Shide E & Chandrasekaran V. The effects of energy drinks on the structure and function of epithelial cells and fibroblasts. 2011; www.stmarys-ca.edu.
9. Kukner A, Colakoglu N, Kara, Oner H, Ozogul C, Ozan E. Ultrastructural changes in the kidney of rats with acute exposure to cadmium and effects of exogenous metallothionein. Biol. Trace Elem Res. 2007; 119(2):137-146.
10. Shimizu S, Eguchi Y, Kamiike W, Waguri S. Uchiyama Y, Matsuda H, Tsujimoto Y. Retardation of chemical hypoxia-induced necrotic cell death by Bcl-2 and ICE inhibitors: Possible involvement of common mediators in apoptotic and necrotic signal transductions. Oncogene 1996; 12: 2045-2050.

Original Article

PRENATAL ZIDOVUDINE INDUCED TOXIC CHANGES IN CEREBELLUM OF SWISS ALBINO MICE

Amit Kumar Nayak *, Shubhangi Yadav, Anand Mishra*, Mandavi Singh***, Vivek Srivastava****, Kapil Malviya*& Chhandamayee Mohanty***

*Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, UP
**Department of Anatomy, Prasad Institute of Medical Sciences, Banthara, Lucknow, UP
***Department of Anatomy, Heritage Institute of Medical Sciences, Bhadwar, Varanasi, UP
****Trauma center, Banaras Hindu University, UP

ABSTRACT

Introduction: AIDS has emerged to be one of the most threatening diseases of 20th century. Zidovudine was the first drug to be introduced for prevention of maternal to fetal transmission. Teratogenic effects of zidovudine are yet to be studied in detail. The aim of the study was to see the effect of various doses of zidovudine on developing swiss albino mice foetus to observe the teratogenic effect on cerebellum.

Material and methods: Zidovudine was given to pregnant mice in the dose of 50, 100 and 150 mg/kg body weight and the cerebellum of 18 day fetus was studied for any gross or microscopic anomaly.

Results: Zidovudine induced a dose dependent damage to the cerebellum of foetus leading to loss of laminar pattern, spongiform appearances and vacuolated spaces due to death of neuronal cells.

Conclusion: Zidovudine is teratogenic and should be cautiously used in clinical scenario.

Key words: Zidovudine, teratogen, HIV-1, AIDS.

INTRODUCTION

Acquired Immune Deficiency Syndrome (AIDS) is a potentially fatal transmissible disease caused by HIV-I virus. Transmission from an infected mother to their infant can take place at three places i.e. in utero, at the time of birth and during breast feeding.

Since the discovery of AIDS in 1981, many drugs have been developed to prevent maternal to fetal transmission. Zidovudine being the first antiretroviral agent to be used in 1987, belongs to the group of antiretroviral drugs called as Nucleoside Reverse Transcriptase Inhibitors (NRTI).

Zidovudine is capable of crossing the feto-placental barrier. Zidovudine is a prodrug and needs to be phosphorylated to its monophosphate by thymidine kinase, followed by a second rate limiting phosphorylation step. This diphosphate is again phosphorylated by enzyme diphosphate kinase into its active form. Although widely used, teratogenic effects of Zidovudine need further investigations.

MATERIAL AND METHODS

Prior approval of institutional ethical committee was taken before the start of the present study. For this study Swiss albino female mice were taken and were kept with male mice for mating overnight in the ratio of 3:1. Presence of vaginal plug was considered to be the first day of gestation (GD 0). The pregnant

Address for Correspondence:
Dr. Amit Kumar Nayak, Assistant Professor, Department of Anatomy, Heritage Institute of Medical Sciences, Bhadwar, Varanasi, UP
Mob: 08726947673 | Email: dramit.k.nayak@gmail.com

female mice were divided into four groups for the present study. The first group was designated as control and was given tap water by gavage from day 6 to 16 of gestation. The other three groups were designated as treated and were given zidovudine in the dose of 50 mg/kg, 100 mg/kg and 150 mg/kg respectively by gavage for the same period. On day 18th of gestation, female mice were sacrificed by cervical dislocation and uterotomy was done to extract the embryos. The brain of the embryos were dissected out and kept in formalin for fixation. For histological study the brain was processed, sections were cut and stained with hematoxylin and eosin.

RESULTS

As compared to cerebellum of control animals which showed well defined outer molecular, middle purkinje and inner granular layers (Fig. 1), treated cerebellum showed dose dependent changes. Low dose (50 mg/kg body weight) treated group showed thinning of molecular layer and spongiform appearance in the interstitial spaces (Fig. 2). Rats treated with 100 mg/kg body weight of zidovudine exhibited thinning as well as loss of cohesion in the cells inside molecular layer. Between the molecular and purkinje layer, a considerable empty spaces were present giving rise to spongiform appearance to the cerebellum. Purkinje and granule cells in the second and third layers respectively were reduced in density (Fig. 3). High dose (150 mg/kg body weight) treated group displayed extreme thinning and degeneration of cells in the molecular layer. The degeneration of cells gave rise to empty vacuolar spaces in the interstitium. Density of purkinje and granule cells was reduced while density of glial cells was increased (Fig. 4).

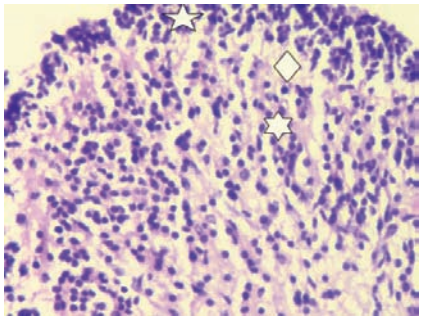

Fig. 1: Photomicrograph of control cerebellar cortex showing well defined molecular (☆), purkinje (◊) and granular cell layer (⇳) in cortex (HE stain, 400x)

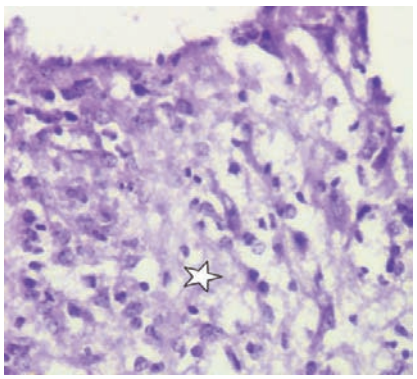

Fig. 2: Photomicrograph of cerebellar cortex treated with 50 mg/kgbw zidovudine showing dilated intercellular spaces with loss of cellularity (☆) (HE stain, 400x)

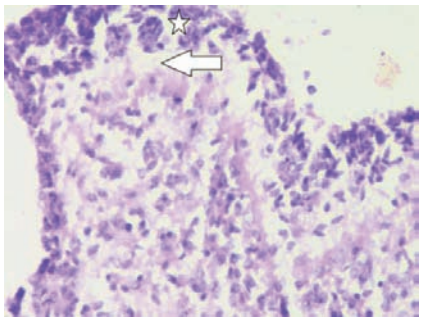

Fig. 3: Photomicrograph of cerebellar cortex treated with 100 mg/kgbw zidovudine showing degenerated molecular (☆) and purkinje cell layer with lacunar spaces (⇦) (HE stain, 400x)

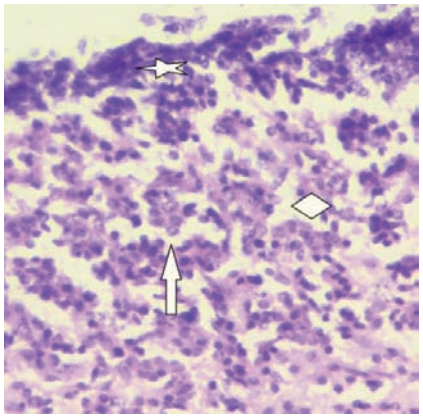

Fig. 4: Photomicrograph of cerebellar cortex treated with 150 mg/kgbw zidovudine showing degenerated molecular layer with cellular debris (☆) in ill defined molecular layer, ill defined purkinje (◊) and granular (⇳) layers showing spongiform appearance (HE stain, 400x)

DISCUSSION

Zidovudine is the first antiretroviral agent to be introduced into the market and is regularly used along with other agents for the treatment of HIV infection and prevention of maternal to child transmission. Zidovudine being a prodrug is first phosphorylated into its biphosphate and triphosphate to be effective [1,2].

The active form of zidovudine mainly affects the viral DNA as well as the mitochondrial DNA of host cells and can cause cell death [3]. When administered during pregnancy it can produce teratogenic effect on the growing embryo specially the nervous system. Ewing et al. (2000) observed reduced NADH in cerebral mitochondria of monkey fetuses exposed to 40 mg zidovuidne per day in utero. The levels of SDH and cytochrome-c reductase were also reported to be elevated. These factors resulted in anomaly of oxidative phosphorylation amounting to mitochondrial damage of cerebellum in a dose dependent manner [4].

Our study also suggests the same as on histological observation of cerebellum there was a dose dependent degeneration of pyramidal cells, edematous change leading to spongiform appearances and finally loss of laminar pattern of cerebellum cortex.

Thus we can say that zidovudine, due to its mitochondrial toxicity induces increased oxygen stress which is toxic to the developing embryo especially in high doses. So, this drug should be used with caution in pregnancy and other compounding factor which can further augment the oxygen stress should be taken care of.

REFERENCES

1. Richman DD, Fischl MA, Grieco MH, Gottlieb MS, Volberding PA, Laskin OL, Leedom JM, Groopman JE, Mioldvan D, Hirsch MS, Jackson GG, Durack DT, Phil D, Nusinoff-Lehrman, S. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: a double-blind, placebo-controlled trial. N Eng J Med. 1987; 317:192-197.
2. Katzung BG, Masters SB, Trevor AJ. Basic & Clinical Pharmacology. McGraw Hill publishers, New York. 11th edition; 427.
3. Sales SD, Hoggard PG, Back DJ. Zidovudine phosphorylation and Mitochondrial Toxicity in vitro. Toxicology and Applied Pharmacology. 2001; 177 (1): 54-58.
4. Moore KL, Persaud TVN .The developing human. 8th ed. Saunders, 2009; 226.
5. Toltzis P, Mourton T, Magnuson T. Effect of zidovudine on preimplantation murine embryos. Antimicrob Agents Chemother. 1993; 37:1610.
6. Ewing Ember L, Gerschenson Mariana. Genotoxic and functional consequences of transplacental zidovudine exposure in fetal monkey brain mitochondria. Journal of Acquired Immune Deficiency Syndrome. 2000.
7. Christmas James T, Little B, Knoll K, Bawdon R. Teratogenic and embryocidal effects of Zidovudine in Sprague-Dawley rats. Infectious Diseases in Obstetrics and Gynaecology. 1995; 2:223-227.
8. Mobarak YM. The in vivo and in vitro developmental toxicity and teratogenicity of the anti AIDS drugs. Trends Appl Sci Res. 2011; 6:208.

A half yearly Journal of Gross Anatomy, Comparative Anatomy, Embryology, Neurology, Histology, Histochemistry, Cytogenetic, Radiological Anatomy, and Allied Clinical Medicine.

Editorial Office:
Dr. Navneet Kumar
Professor & Head
Department of Anatomy
KGMU, Lucknow,-226003

Anuj Printers Lucknow