ISSN 2518 - 6876 (Print)
Bib ID : 2570180

Volume 2 Issue 2
July 2016

North Bengal Medical College Journal

Contents

Official Organ of
North Bengal Medical College, Sirajganj

NORTH BENGAL MEDICAL COLLEGE JOURNAL

Vol 2 **No 2** **July 2016**

Contents

An Official Organ of North Bengal Medical College, Sirajganj

NORTH BENGAL MEDICAL COLLEGE JOURNAL

Vol 2 No 2 July 2016

The North Bengal Medical College Journal (NBMC J) is a peer-reviewed journal published biannually. It is the official organ of North Bengal Medical College, Sirajganj, Bangladesh.

Address of Correspondence:

Editor in Chief
North Bengal Medical College Journal
North Bengal Medical College
Dhanbandhi, Sirajganj.
Email: thenbmcj@gamil.com

Annual subscription

Taka 300/= for local subscribers

USD 20 $ for overseas subscribers

Editorial

PowerPoint for Teaching in Classroom

Preamble

We may have many years of classroom experience, as a student and as a teacher, which guides our teaching. However, we are less likely to have had similarly rich experiences with instructional technologies, as these tools have become available only more recently. There are many tutorials, books and other resources for using presentation technologies, like PowerPoint. However, most deal only with the mechanics of creating slides and presentations and of good design from a graphic perspective. There is very little about effective teaching with PowerPoint.

We mostly practice simply convert our lecture notes and transparencies from text into PowerPoint slides. However, the researches indicate that this may be possible to do it more effective, if we expand the possibilities and scopes of the methodology. A number of educational models could be applied to enhance PowerPoint presentation but we have to remember that are some thumb rules, which are common for all PowerPoint technologies for teaching and learning.

PowerPoint is an aid for teaching, not the content. So we should remember goals of PowerPoint beforehand:

(1) Gaining attention of the students in the class;
(2) Inform the learner of the lesson objectives first;
(3) Recall of prior learning (without slide);
(4) Eliciting performance and then start the real PowerPoint presentation.

We should keep the point in mind that the learner may not be well to do with PowerPoint before; so provide them opportunity to be familiar. Students can be asked to respond by offering several examples. The repetition increases the likelihood of retention. So, don't be panic or don't go fast or try to avoid.

Potential benefits

(1) Engaging multiple learning styles; (2) Increasing visual impact; (3) Improving audience focus; (4) Enriching curriculum with inter-disciplinarily; (5) Increasing spontaneity and interactivity and (6) Increasing wonder.

Assessment of the Situation

Think that you are in an average classroom today and you will find a group of students, sitting at their desks, eyes glazed over as their teacher stands in front of a projected screen. The teacher, frequently with a laser pointer, will be directing students to look at images, text, or videos projected on that screen. They will drone on, often with their back to the class, reading long blocks of text. Some students will fall asleep, while

others will simply zone out. At the end of the class the bell will ring, students will spring into action, eager to leave, having learned nothing.

This horror story, sorry to say, we have witnessed from both sides of the projector. Then I asked the question to myself – is the result of teachers incorrectly using PowerPoint, a computer program which launched in 1990? The answer is certainly "No". This happens due to our lacking of emphasis and improper knowledge of human behavior as well using PowerPoint. Some points should be in consideration, when we use PowerPoint.

Best PowerPoint Techniques:

(1) Text size: text must be clearly readable. (tips: Size in yards of the room will be the minimum font-size);
(2) Avoid too much text. (Follow 6x6 rules; i.e., no more than:- 6 words per line; 6 lines per slide; 36 numbers per slide);
(3) No slide should contain more than one-theme; not more than 2 illustrations per slide.
(4) Contrast: light text on dark backgrounds will strain the eyes. Minimize this contrast;
(5) Avoid red-green combination (because many people are color-blind);
(6) Transitions should be used cautiously and consistently (to avoid distractions);
(7) Animation should not be the attraction, rather essential.
(8) Template must not change often; and
(9) Graphics and pictures should use to enhance the text, not just for prettiness.

Using Signs:

(1) Use less sub-blocks;
(2) Reveal bullet points or table rows one at a time (so that the last one visible is the one you are talking about);
(3) Use arrows, circles or other pointers to show what you are referencing in specific parts of an illustration, photo or graph;
(4) Animate and reveal parts of illustrations and graphs (where possible) to build your story rather than showing everything at once and
(5) Use bold type or different colors to highlight the keywords in any lengthy text.

Avoid while presenting :

(1) Thorough reading;
(2) Quick transition;
(3) Change font frequently (by size, color or alignment);
(4) Shapes;
(5) Clumsiness and
(6) Too burden-figure or huge table.

Smart Keys: There are some keystrokes (shortcuts) we can use while presenting, which make a presenter more elegant. We can mention here few of them:

(1) Next slide =N;
(2) Previous slide =P;
(3) Go to slide "number“ ="number"+Enter;
(4) Black screen =B;
(5) White screen =W;
(6) Change pointer to a pen =CTRL+P;
(7) Change pen back to arrow =CTRL+A;
(8) Erase onscreen annotation =E;
(9) End slide show =ESC, etc.\

Last Word: Make your presentation lively, easy and possibly a-beat funny. There's little doubt that emotional responses can aid memory. However, remember, the point of presentation slides is not to replace you as the teacher, but to help your students understand the subject and remember what you are teaching. Overwhelming them with too much information can be just as harmful as underwhelming them with too little.

Courtesy :

Prof. Khondaker Manjare Shamim

Department of Anatomy, BSMMU, Dhaka.

Prof. Khondaker Bulbul Sarwar
Professor of Community Medicine
North Bengal Medical College, Sirajganj
<kbsarwar@gmail.com>

References

1. Brown JS, Collins A, Duguide S. Situated cognition and the culture of learning. Educ Res. 1989;18(1): 32–42
2. Keller J. Killing me Microsoftly with PowerPoint. 2003 Chicago Tribune, January 5.
3. Dede C. Emerging technologies and distributed learning. Am J. Distance Educ. 1996;10(2): 4–36.
4. Dede C, Whitehouse P, Brown-L'Bay T. Designing and studying learning experiences that use multiple intera-ctive media to bridge distance and time. In: Vrasidas C, Whitehouse C, Glass G, editors. In: Current Perspectives on Applied Information Technologies. (Volume 1: Distance Education) Greenwich, CT: Information Age Publishing; 2002.
5. Thompson C. PowerPoint makes you dumb. 2003 New York Times, December 14.
6. Lave J, Wenger E. Situated Learning: Legitimate Peripheral Participa-tion. Cambridge, United Kingdom: Cambridge University Press; 1991.
7. Trotter A. Question of effective-ness. Education Week. 1998;18(5): 6.
8. Parks, Bob (2012-08-30), "Death to PowerPoint!", Bloomberg Businessweek, businessweek.com, retrieved 6 Septem-ber 2012 from Wikipedia.

Instructions for Authors

Authors are invited for submission of articles in all fields of medical science and all correspondence, should be addressed to

Editor in Chief,

North Bengal Medical College Journal,

North Bengal Medical College and Hospital,

Dhanbandhi, Sirajganj.

Email: thenbmcj@gamil.com

Overall general Instructions

- Type manuscripts in British English in double-spaced paragraph including references, figures with legends and tables on one side of the page.
- Leave 2.5 centimeter margin on all sides with number in every page at the bottom of the page (middle, by page/ x or y) beginning with the abstract page and including text, tables, references and figures.
- Cite each reference in text in Arabic numbers (1, 2, 3, ...) numerical order with their lists in the reference section (as Vancouver Style).
- SI units of measurement should be used.
- Assemble manuscript in following order :

  (1) Title page;
  (2) Then next page with author designations and place of work.
  (3) Abstract (structured) within 250 words.
  (4) Main text which includes Introduction, Materials and methods, Results, Discussion, Conclusion, Acknowledgments (if any) and contributions of the authors in that specific study.
  (5) References;
  (6) Tables;
  (7) Figures with legends

- You can follow recommendations of ICMJE for muscript perparation at www.icmje.org.
- Articles should not exceed over 10,000 words**.** Over–length manuscripts will not be accepted for publication.
- Submit two copies of the manuscripts with electronic version (MS word) which is needed to be submitted in a compact disc.

First title page with author information (1st page should not be numbered). Please make abstract page, with title of the article without authors name to make it anonymous for review.

Text page must include:

- Full title of the article not exceeding 50 characters with three to five key words for use as indexing and a running title for use on the top of text pages.

- Authors' names, highest academic degree, affiliations and complete address for correspondence and they should reveal all possible conflicts of interest on this page.
- **Abstract page (First numbered page)**
- Do not cite references in the abstract (250 words, maximum).
- Limit use of acronyms and abbreviations. Abbreviations must be defined at the first mention.
- The abstract should cover ***Background and Purpose*** (description of rationale for study); Methods (brief description of methods); Results (presentation of significant results) and conclusion (succinct statement of data interpretation) in a running manner and not under separate headings.

The Text

The Following are typical main headings:

i. **Introduction**
ii. **Materials and Methods**
iii. **Results**
iv. **Discussion and Conclusion.**

Introduction:

Summarize the rationale for the study with pertinent references. The purpose (s) of the study should be clearly elicited.

Materials and Methods:

Identify type of study and describe the study subjects and methods used with methods of statistical analysis. Cite reference (s) for standard study and statistical methods. Describe new or modified methods. Give proper description of the apparatus (with name and address of manufacturer) used. ***Generic name of drug must be given. Manuscripts that describe studies on humans must indicate that the study was approved by an institutional Ethical Committee and that the subjects gave informed consent.***

Results:

Present only important results observations in logical sequence in the text, tables or illustrations with relevant statistics.

Discussion:

Emphasize new and important results and the conclusions that follow including implications and limitations. Relate observations to other relevant studies.

Conclusion:

Link the conclusion with the goals of the study, but avoid unqualified statements and conclusions not adequately supported by data. State new hypothesis when warrented.

Acknowledgments:

List all sources of funding for the research with contributions of individuals.

References:

Accuracy of reference data is the author's responsibility. Verify all entries against original sources. Especially journal tittles, inclusive page numbers, publication dates. All authors must be listed if less than six. Use et al, if more than six. Personal communications, unpublished observations, and submitted manuscripts must be cited in the text as "([Name(s)], unpublished data, 20xx)." Abstracts may be cited only if they are the sole source and must be identified in the references as "Abstract". "In press" citations must have been accepted for publication and add the name of the journal or book including publisher. Use Vancouver style, for example:

1. World Health Organization: WHO Recommendations: Low Birth Weight: preventing and managing the Global Epidemic. Geneva, World Health Org, 2000 (Tech.Rep.Ser., no.894).
2. Rashid M. Food and Nutrition. In : Rashid KM, Rahman M, Hyder S, eds. Textbook of community Medicine and Public Health. 4th edn. RHM Publishers: Dhaka. 2004; p. 156-160.
3. Arefin S, Sharif M, Islam S. Prevalence of pre diabetes in a shoal population of Bangladesh. BMJ. 2009; 12: 155-163.
4. Jarrett RJ. Insulin and hypertension (Letter). Lancet 1987; ii: 748-749.
5. Reglic LR, Maschan RA: Central obesity in Asian men. [Abstract]. J Clin Endocrinol Metab. 2001; 89: 113-118.
6. Hussain MN, Kamaruddin M. Nipah virus attack in South East Asia: challenges for Bangladesh. [Editorial]. Prime Med Coll J. 2011; I (1): i-ii.

Tables:

Each Table must be typed on a separate page. The table number should be followed by a Roman brief informative title. Provide explanatory matter in footnotes. For footnotes use symbol in this sequence; *, **, +, ++, etc.

Figures:

Line drawings, photomicrographs, colour prints and halftones should be camera ready, good quality prints. Submit only originals of laser prints, not photocopies. Original figures must be submitted indicating figure number, short figure title on top of figure lightly in pencil. Any abbreviation or symbols used in the figures must be defined in the figure or figure Legend.

North Bengal Medical College Journal
Vol 2 No 2 July 2016

Original Article

Morphometric Study of Foramen Magnum of Adult Dry Skull

SM Akram Hossain,[1] A S M Ashrafuzzaman,[2] Pervez Shake[3]

Revised : January 20, 2016 Accepted : April 03, 2016

Abstract

Introduction: *The foramen magnum is a large opening in the occipital bone of cranium and is a unique and complex neuroanatomical structure. This is an important landmark of skull base for neurosurgical procedures. The dimensions of the foramen magnum are clinically important because vital structures passing through it may endure compression such as in cases of foramen magnum herniation, foramen magnum meningiomas and foramen magnum achondroplasia. So, the aim of this study was to analyze the morphometry pertaining to comparing antero-posterior and transverse diameters available in literature and to bring out associated clinical implications.*

Methods: *In this cross sectional study, one hundred thirty eight dry, adult human skull of unknown sex were examined and measured antero-posterior and transverse diameters with the help of Vernier-caliper. Additionally, surface area of foramen magnum was also calculated.*

Results: *The mean antero-posterior diameter of the foramen magnum was 31.3 mm (range 20.0-41.2mm) and the transverse diameter was 26.92 mm (range 19.2-36 mm). The mean surface area of foramen magnum was 674.7 mm^2. The mean of foramen magnum index was 86.31%.*

Conclusion: *The dimensions of foramen magnum have been evaluated in the Bangladeshi population for the first time. This analysis will be of paramount importance for skull base surgery and also helpful for radio-imaging diagnosis. Considering the above mentioned importance, this study is worthwhile.*

Key words: *Foramen magnum, Skull base, Morphometry, Sexual dimorphism*

North Bengal Med. Coll.J. 2016; 2 (2) : 07-13

1. Professor and Head, Department of Anatomy, North Bengal Medical College, Sirajganj
2. Lecturer in Anatomy, North Bengal Medical College, Sirajganj
3. Lecturer in Anatomy, North Bengal Medical College, Sirajganj

Correspondence *S M Akram Hossain, Email: akhossain_09@yahoo.com*

Introduction

The Foramen Magnum (FM) in Latin: 'great hole' is a large opening in the occipital bone of the cranium and it is an important landmark in the posterior part of the cranial base. Its transverse diameter is rather less than one third of the distance between the mastoid processes. The anterior border of the foramen magnum is formed by the basilar process of the occipital bone, the lateral border by the left and right ex-occipitalis and posterior border is formed by the supra-occipital part of occipital bone.[1] It lies in an antero-median position and leads into the posterior cranial fossa. It transmits the lower end of the medulla oblongata, meninges, vertebral arteries and spinal accessory nerve; the apical ligament of the dens and the tectorial membrane pass through it to the internal basiocciput. Anteriorly, the margin of the foramen magnum is slightly overlapped by the occipital condyles which project down to articulate with the superior articular facets on the lateral masses of the atlas.[2] A fundamental knowledge of the normal anatomy of the cranial base, specially the foramen magnum and associated structures, is important to the clinician for accurate diagnosis and treatment of various diseases.[3]

The dimensions of FM have clinical importance because the vital structures that pass through it may suffer compression such as in cases of FM achondroplasia[4] and FM brain herniation.[5,6] These may result into life-threatening respiratory complications, lower cranial nerve palsies, and paresis of upper and lower extremities. In a computerized tomographic study of Catalina & Herrera, dimensions of the foramen magnum of 63 achondroplastic individuals were compared to standards established for non-achondroplastic individuals. The size of the foramen magnum in patients with achondroplasia was small at all ages, particularly in those with serious neurological problem.[7] In neurosurgical practice, the transcondylar approach is commonly used to access the lesions which are ventral to the brainstem and cervicomedullary junction. It was reported that understanding the bony anatomy of the condylar region is important for this approach.[8] Furthermore, it was stated that longer FM antero-posterior dimensions permitted greater contralateral surgical exposure for condylar resection.[9] The knowledge of foramen magnum diameters is needed to determine some malformations such as Arnold Chiari syndrome, which shows expansion of transverse diameter.[10] It can be used in the field of forensic identification and anthropology for determination of the gender of human skulls.[11-13] This knowledge can be applied in its morphometric analysis to determine sex

in medicolegal purpose, when there is involvement of other parts of the craniofacial skeleton, as in severe injuries, aircraft accidents, fire or explosion.[14,15]

It is obvious that, FM evaluation was very important to ascertain the appropriate surgical techniques, and also to obtained the useful data for unknown sex determination and age estimation for medicolegal purpose. So, the aim of the present study was to evaluate the dimensions of foramen magnum.

Materials and Method

This is a cross-sectional type of study. In this study the samples were included by random collection of 138 adult human dry skulls from departments of anatomy, North Bengal Medical College, Sirajganj, Shahid M. Mansur Ali Medical College, Sirajganj, Barind Medical College, Rajshahi and Rajshahi Medical College, Rajshahi. The skulls that have been damaged, eroded, deformed and those of children were excluded from the study. They were used for tutorial teaching for medical students. With the help of simple Vernier-caliper antero-posterior (**APD**) and transverse diameters (**TD**) of foramen magnum were measured. The length of foramen magnum was measured from the anterior border (basion) through the center of the foramen magnum until the end of the posterior (opisthion). The transverse diameter was measured from the point of maximum concavity on right and left margins (Figure 1).

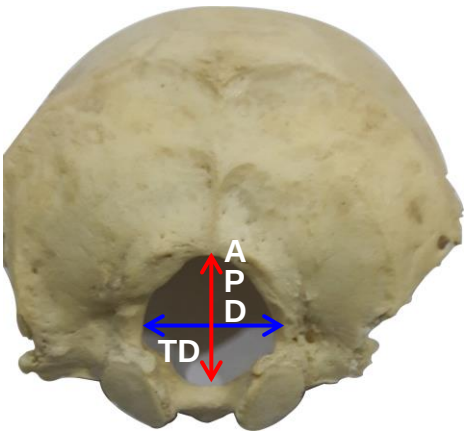

Figure 1 : Measurement of diameters of Foramen Magnum in Skull

The area of foramen magnum (FMA) was calculated using formula derived by Randinsky.[16] Surface area of foramen magnum was calculated by using formula stated below.

$$\text{AREA} = 1/4 \times \pi \times h \times w$$

Where, π (mathematical constant) = 22/7 (3.14)

h= antero-posterior diameter

w= transverse diameter

Foramen magnum index (FMI) was calculated by: Foramen magnum width (TD) × 100/ Foramen magnum length (APD).

Results

In this study, the dimensions and surface area of foramen magnum are shown in Table-I.

Table I: Dimensions and surface area of Foramen Magnum

Values	Antero-Posterior diameter (APD) (mm)	Tansverse diameter (TD) (mm)	Surface area (mm^2)
Maximum	41.2	36.0	1015.5
Minimum	20.0	19.2	329.7
Mean	31.3	26.9	674.7

After proper verification regarding consistency and validity, data were entered into computer by using SPSS 16 version programme. Statistical significance was found by applying relevant statistical test at appropriate probability level ($p<0.01$).

The mean dimension of foramen magnum in APD was 31.3 mm, whereas it was 26.9 mm in TD. APD of foramen magnum had highly significant in compared to TD ($p<0.01$).The mean surface area of foramen magnum was 674.7 mm^2 (range 329.7 mm^2-1015.5 mm^2). Mean foramen magnum index (FMI) was 86.31% (range 65.29% -109.15%).

Discussion

Foramen magnum is morphologically variable osteological feature in the skull which has undergone evolutionary changes.[17] The dimensions of the foramen magnum are clinically important because vital structures passing through it. In the present study the average antero-posterior diameter (APD) of the foramen magnum was 31.3 mm (range 20.0-41.2 mm) and transverse diameter (TD) was 26.9 mm (range 19.2-36 mm) respectively. The mean surface area of foramen magnum was 674.7 mm^2. Other researchers were observed that the average APD of the foramen magnum was 33.3 mm (range 27-39 mm) and the TD was 27.9 mm (range 23-32 mm).[8] There is statistically significant difference between present study and observation done by previously reported by researchers ($p<0.01$). It was found that the mean APD was 3.1cm, and mean TD was 2.7cm and mean surface area of the foramen magnum was 558 mm.[2,18] In Catalina-Herrera's[7] anatomic study of FM, the diameters were 35.2 mm for APD and 30.3 mm for TD and the means of the FM in male and female skulls were 888.4 mm^2 and 801 mm^2 respectively.

It was reported by Berg and Bergmann in their study, the average APD of 34 mm and TD of 29 mm.[19] A study was conducted on skulls of Karnataka showed that, the mean APD in male was 33.4 mm, female was 33.1 mm and by CT imaging method in male was 38.5 mm and female was 35.2 mm. The mean TD of FM in male was 28.5 mm and female was 27.3 mm but by CT imaging method in male was 29.1 mm and female was 27.6 mm.[15] In Western Europe, a study was carried on skulls confirmed that APD

ranges 30 mm to 43 mm with the mean of 36.6 mm but TD ranges from 25 mm to 39 mm with the mean of 31.1 mm.[20] Similarly, another study on Brazilian individuals in relation to gender established that mean APD was 35.7 mm in male and 35.1 mm in female but the TD was 30.3 mm in male, 29.4 mm in female.[14] In a cadaveric CT images measurements conducted by Wanebo & Chicoine[9] showed that, mean area of the FM is 820.0 ± 100.0 mm^2, the mean length (SD) 36.0 ± 2.0 mm and the mean width (TD) 32.0 ± 2.0 mm. A study was conducted on fifty-four cranial CT scans obtained from the archives of Department of Radiology and observed that mean APD of FM was 35.58 mm and TD was 29.84 mm. The mean APD in male and female was 30.75 mm and 29.98 mm respectively. The mean TD in male and female was 36.95 mm and 34.41 mm respectively. There was a significant difference between the APD of male and female cases.[21] There was a significant difference in mean of FM area among male and female ($p<0.001$) which were 909.91 mm^2 in males, 819.01 mm^2 in females.[22] Our study revealed the mean foramen magnum index (FMI) was 86.31% whereas in a Brazilian study conducted by Pires et al.[23] it was 83.75%.

As it has been mentioned, the FM includes specific neuroanatomic structures[24-27] and lesions occupied in that area needs special microsurgical intervention.[27] A meticulous planning mainly based on the FM sizes is very essential for choosing and establishing the most appropriate surgical techniques to refrain from any neurological impairment.[27,28] In addition, it is quite difficult to detect many pathological lesions not only by neurological examination but also needs support from the radiological findings.[23,27]

Conclusion

The knowledge of diameters of the foramen magnum are needed to determine the radiological malformations (Arnold Chiari's syndrome) and prior to cutting off of foramen magnum or posterior cranial fossa lesions, or sex determination of skulls medico-legal purpose. So, the knowledge of dimensions of foramen magnum are important for neurosurgeons, radiologist as well as anthropologists.

Acknowledgements

We are very much grateful and express our deep gratitude to the authority of North Bengal Medical College Sirajganj, Shaheed M. Monsur Ali Medical College, Sirajganj, Barind Medical College, Rajshahi and Rajshahi Medical College, Rajshahi, for their cordial supports during sample collection.

Contribution of the Authors

The first author was the principal researcher, while the second and third were involved in data collection, computer composing and data analysis.

References

1. Scheuer L, Black S. The juvenile skeleton. Elsevier. London 2004;p1-19.
2. Standarding S. Gray's anatomy. The anatomical basis of clinical practice. 39th ed. London: Elsevier Churchill Livingstone, 2005;p.460.
3. Gautam K, Vijay P, Yad RY, Pushp RB, Dhananjay S. Morphometric analysis of posterior fossa and foramen magnum. J Neurosci Rural Pract. 2012;3(3): 261-266.
4. Hecht TJ, Horton WA, Reid CS, Pyeritz RE, Chakraborty R. Growth of the foramen magnum in achondroplasia. AJMG. 1989;32: 528-535.
5. Reich JB, Sierra J, Camp W, Zanzonico P, Deck MD, Plum F. Magnetic resonance imaging measurements and clinical changes accompanying transtentorial and foramen magnum brain herniation. Annals of Neurology. 1993;33: 159-170.
6. Ropper AH. MRI demonstration of the major features of herniation. J Neurol Neurosurg Psychiatry. 1993;56: 932-935.
7. Catalina-Herrera CJ. Study of the anatomic metric values of the foramen magnum and its relation to sex. Acta Anat. 1987;130: 344-347.
8. Muthukumar N, Swaminathan R, Venkatesh G, Bhanumathy SP. A morphometric analysis of the foramen magnum region as it relates to the transcondylar approach. Acta Neurochir (Wien). 2005;147: 889-895.
9. Wanebo JE, Chicoine MR. Quantitative analysis of the transcondylar approach to the foramen magnum. Neurosurgery. 2001;49: 934-941.
10. Sgouros S, Goldin JH, Hockely AD, Wake MJ, Natarajan K. Intracranial volume change in childhood. J Neurosurg. 1999;91: 610-616.
11. Tanuj K, Anadi G, Kewal K. Craniometric analysis of foramen magnum for estimation of sex. Int J Med, Health, Biomed and Pharm Eng. 2013;7(7): 111-113.
12. Suazo GIC, Russo PP, Zavando MDA, Smith RL. Sexual Dimorphism in the foramen magnum dimensions. Int J Morphpol. 2009;27(1): 21-23.
13. Edwards K, Viner MD, Schweitzer W, Thali MJ. Sex determination from foramen magnum. J Forensic Radiol and Imaging. 2003;1(4): 186-192.
14. Manoel C, Prado FB, Caria PHF, Groppo FC. Morphometric analysis of the foramen magnum in human skulls of brazillian individuals: its relation to gender. Braz J Morphol Sci. 2009;26(2): 104-108.
15. Muralidhar P, Magi M, Nanjundappa B, Pavan PH, Premalatha G, Shaik HS.

Morphometric analysis of foramen magnum. Int J Anat Res. 2014;2(1): 249-255.

16. Randinsky L. Relative brain size is a new measure. Science. 1967;155: 836-838.
17. Nevell L, Wood B. Cranial base evolution within the hominin clade. J Anat. 2008;212: 455-468.
18. Tubbs RS, Griessenauer CJ, Loukas M, Shoja MM, Cohen-Gadol AA. Morphomretric analysis of the foramen magnum: an anatomic study. Neurosurgery 2010;66(2): 385-388.
19. Berg JK, Bergmann RA. Variation in size and in symmetry of the foramina of the human skull. Clin Anat. 2001;14: 406-413.
20. Gruber P, Henneberg M, Boni T, Ruhli FJ. Variability of human foramen magnum size. Anat Rec. 2009;292: 1713-1719.
21. Erdil FH, Sabancyooullary V, Cimen M, Ipyk O. Morphometric analysis of the foramen magnum by computed tomography. Erciyes Med J. 2010;32(3): 167-170.
22. Gunay Y, Altinkok M. The value of the size of foramen magnum in sex determination. J Clin Forensic Med. 2000;7(3): 147-149.
23. Lucas AS Pires, Álvaro R Teixeira, Tulio FO Leite, Marcio A Babinski, Carlos AA Chagas. Morphometric aspects of the foramen magnum and the orbit in Brazilian dry skulls. Int J Med Res Health Sci. 2016;5(4): 34-42.
24. Williams PL, Warwick R. Gray's Anatomy, 7th Ed. New York: Churchil Livingstone. 1989;p.342-361.
25. Snell RS. Clinical Anatomy for Medical Student, 4th edition. Boston Little, Brown and Company. 1992;p.808-812.
26. De Oliveira E, Rhoton AL Jr, Peace D. Microsurgical anatomy of the region of the foramen magnum. Surg Neurol. 1985;24: 293-352.
27. Coin CG, Malkasian D R. Foramen magnum. In Newton TH, Potts DG, editors. Radiology of the Skull and Brain: The Skull. Vol. 1, book 1 St. Louis: Mosby. 1971;p.275-286.
28. George B, Lot G, Boissonnet H. Meningioma of the Foramen Magnum: a series of 40 cases. Surg Neurol. 1997;47: 371-379.

North Bengal Medical College Journal
Vol 2 No 2 July 2016

Original Article

Effects of Enlarged Adenoids on Middle Ear Pressure and Hearing

Md. Saber Ali,[1] Md. Enamul Haque[2]

Revised : January 08, 2016 Accepted : April 09, 2016

Abstract

Introduction: *Enlarged adenoid was diagnosed in 52 (1.15%) cases of children under15 years of age with variable size reducing nasopharyngeal space to a various degree. Relative size of adenoid or its extension laterally may obstruct pharyngeal opening of eustachian tube resulting significant changes to negative middle ear pressure and hearing impairment. The aim of this study was to assess the morbidity in children and adolescent resulting from unwanted effects of enlarged adenoid on middle ear pressure and hearing.*

Methods: *This study was done to indentify middle ear pathology. Patients were from out patient department of Rajshahi Medical College Hospital, January to December 2004. Total 52 patients were included both male and female sexes of age ranging from 3-15 years belong to different socio-economic conditions, children of middle socio-economic status taking unbalanced diet, living overcrowded on kacha floor shown higher incidence of enlarged adenoids. Patients were diagnosed as having enlarged adenoid from history, clinical examination & X-ray nasopharynx lateral view was done in all cases. Tuning fork test, pure tone audiometry and impedance audiometry (Tympanometry) were also done.*

Results: *Significant number of cases 27 (51.92%) having hugely enlarged adenoid between age group 6-10 years were observed.*

Conclusion: *This study was done to develop awareness about the effects of enlarged adenoid on hearing and speech by proper health education and primary health care measures. It reduces the risk of orthodontic architectural deformity by early diagnosis and treatment.*

Key words: *Adenoids, Middle ear pressure, Hearing*

North Bengal Med. Coll.J. 2016; 2 (2) : 14-22

1. *Associate Professor, Ear, Nose and Throat Department, North Bengal Medical College, Sirajganj*
2. *Assistant Professor, Ear, Nose and Throat Department, Rajshahi Medical College, Rajshahi*

Correspondence *Md. Saber Ali, Email: saberalient@gmail.com*

Introduction

Nasopharyngeal tonsils are three in number, one of them is situated in the midline at the junction of posterior pharyngeal wall and roof of the nasopharynx. When that is enlarged and produces symptoms, then it is regarded as adenoid.[1] Mildly enlarged adenoid means soft tissue shadow occupying less than $^{1}/_{3}^{rd}$ of the space in nasopharyngeal air column. Moderately enlarged adenoid occupies more than $^{1}/_{3}^{rd}$ but less than $^{2}/_{3}^{rd}$ space in the nasopharyngeal air column. But hugely enlarged adenoid refers to a size more than $^{2}/_{3}^{rd}$ space is occupied by soft tissue shadow.[4] Others two are tubal tonsils placed at posterior aspect of pharyngeal openings of eustachian tubes connecting middle ears to nasopharynx. Lateral extension or enlargements of adenoids produce nasal and / or aural symptoms due to reduction of nasopharyngeal space and / or obstruction to pharyngeal opening of eustachian tube. Commonly affected group is children and adolescent age ranging from 3 to 15 years with insignificant male: female ratio. Variable degree of nasopharyngeal space reduction from various degrees of enlarged adenoids and air column versus soft tissue shadow ratio measured by radio-image study. Nasopharyngeal space measured from basi-occiput to soft palate of its narrowest part. Various degree of adenoids enlargement measured by Adenoidal: Nasopharyngeal space ratio.[10]

Ill effects of enlarged adenoid on middle ear pressure and different pathological changes as otitis media with effusion (OME), chronic supporative otitis media (CSOM) even persistent residual changes in tympanic membrane. The aim of this study is to reduce morbidity in children and adolescent resulting from unwanted effects of enlarged adenoids on middle ear pressure and hearing. This affects speech development, social communication, education and impaired mental growth. It also causes orthodontic deformity due to nasal breathing obstruction.

Material and Methods

All children age between 3 to 15 years, were collected from out patient department (OPD) of Ear, Nose and Throat (ENT) department of Rajshahi Medical College & Hospital from January to December 2004 and diagnosed as having enlarged adenoid through a prescribed protocol by proper history taking and physical examinations. All cases X-ray nasopharynx lateral view and others relevant investigations were done. Middle ear status was assessed by Tuning Fork test, Pure-tone audiometry (PTA), impedance audiometry (Tympanom-etry) and pathological changes were correlated with degree of adenoidal enlargement. Pressure changes assessed by Tympanometry, normal intra-tympanic pressure is -100 mm of H_2O to +50 mm of H_2O, below -100 mm of H_20 to -200 mm of H_2O will be regarded as negative middle ear pressure. Normal compliance is 0.39 ml – 1.30 ml. Hearing impairment assessed by Tuning Fork test but its degree of hearing

loss measured by PTA. Mild hearing loss is labeled as 30 deciBell (dB) to 45 dB. Moderate hearing loss means 46 dB to 60 dB but severe loss is 61 dB to 90 dB. A patient described as totally deaf with hearing loss above 120 db which is beyond the maximum output of an audiometer. Tuning Fork test assess qualitative aspect indicated by Rinne's test negative and weber's test lateralised to the affected ear, patient having conductive hearing loss.

Results

Out of 4550 children under 15 years age ranging between 3-15 years were examined in ENT, out patient department (OPD) of Rajshahi Medical College and Hospital. Out of them 52 were diagnosed as having enlarged adenoid, produced various symptoms most common was open mouth breathing and others shown in Table I.

Table I: Presenting symptoms with number and percentage of Patients (n–52)

Symptoms	Number of patients	Percentage
Mouth breathing	34	65.38
Hearing impairment	27	51.92
Snoring	23	44.23
Nasal blockage and discharge	20	40.38
Dribbling of saliva	18	34.61
Persistent ear discharge	13	24.99
Bed wetting	10	19.38
Sleep disturbance	08	15.38
Epistaxis	06	11.53
Voice change and cough	06	11.52
Headache	05	09.61

Presence of various sizes of adenoidal enlargement with their percentage distribution is shown in Table II.

Table II: Different sizes of enlarged Adenoids and their percentages (n - 52)

Sizes of Adenoids	Numbers	Percentage
Hugely enlarged	27	51.92
Moderately enlarged	22	42.31
Mildly enlarged	03	05.77

Typical adenoid facies was found in one male patient (1.92%). Age and sex distributions were studied and found 28

(53.84%) in male and 24 (46.16%) in female children. But the age range varies from 3-15 years, among which 10 (19.23%) were between 3-5 years, 35 (67.31%) between 6-10 years, and 7 (13.46%) were above 10 years (Table III).

Table III: Age and sex distribution of Patients (n-52)

Age in years	Sex		Total	Percentage
	Male	Female		
	28	24		
3-5 Years	05	05	10	19.23
6-10 Years	19	16	35	67.31
Above 10 Years	04	03	07	13.46

Male: female ratio with prevalent age group is 1.66: 1.

In this study, clinically it was revealed that, middle ear pathology were in the form of OME 62 (59.62%), CSOM 26 (24.99%) unilaterally or bilaterally or combined in the same patient. There was overlapping found in patient of OME in one ear and CSOM in other ear (Table IV).

Table IV: Percentage of various Middle Ear Pathology (n – 104 ears)

Clinical diagnosis	Number of ear affected	Percentage of ear affected
OME (otitis media with effusion)		
Bilateral	32	30.78
Right Ear	16	15.38
Left Ear	14	13.46
Total	**62**	
CSOM		
Bilateral	14	13.47
Right Ear	07	06.73
Left Ear	05	04.80
Total	26	
Normal Ear	16	19.59
Total	**104**	

Of the OME majority showed mild to moderate hearing loss ranging from 40-60 dB. Pure-tone Audiometry changes were correlated with clinically diagnosed

conditions. Test (PTA) was not done in 6 cases (12 ears). In Clinical findings, PTA and Tuning Fork test were correlated in Table V.

Table V: Correlation of clinical findings, PTA and Tuning fork tests (n-104)

Ears Involved	Suspected hearing loss on clinical findings (n-104)	Hearing loss found by pure-tone Audiometry (n-92)	Normal hearing by Audiometry test (n-92)	Hearing loss found by Tuning Fork test (n-92)
Bilaterally affected Ears	44 (42.31%)	40 (43.48%)	8 (8.69%)	20 (21.73%)
Right Ear	23 (22.12%)	20 (21.74%)	4 (4.35%)	14 (15.21%)
Left Ear	21 (20.19%)	16 (17.39%)	4 (4.38%)	10 (10.86%)
Total	**88 (84.62%)**	**76 (82.61%)**	**16 (17.39)**	**44 (47.8%)**

Ears with intact but abnormal looking retracted ear drum and a few normal looking drum were mostly subjected to impedance audiometry and flat curve obtained in 44 (66.66%) ears, rest showing normal tympanogram 22 (33.34%). Tympanometric abnormality indicating pressure changes in the middle ear cavity. Pressure changes observed in 50 (75.76%) ears of which 46 (69.69%), showing negative pressure 4 (6.07%) showing positive pressure and 16 (24.24%) has shown normal pressure. Six cases were dropped out and clinically established CSOM also excluded from Tympanometric investigations. As the pressure gradient could not be created due to lack of sealing mechanism, the test results correlated in Table VI.

Table VI: Correlation between clinical and tympanometric findings (n – 66)

Total number Ears (n -104)	Clinically abnormal ear drum (TM)		Impedence audiometric Curve
	Intact - 62	Perforated - 26	Flat-Curve - 44
Bilateral	32 (30.78%)	14 (13.47%)	22 (33.33%)
Right	16 (15.38%)	7 (6.73%)	12 (18.18%)
Left	14 (13.46%)	5 (4.8%)	10 (15.15%)

Clinically in 16 (15.38%) cases, normal tympanic membrane (TM). Were observed Pressure changes in the middle ear were observed by Tympanometry (Table VII).

Table VII: Tympanometry showing pressure changes in the middle ear (n-66)

Tympanometric pressure change	Numbers of ears	Percentage	Total numbers of ears affected
Negative pressure 46	Bilateral = 24	36.36%	75.76%
	Right = 12	18.18 %	
	Left = 10	15.15%	
Positive pressure 4	Bilateral = 2	3.03%	
	Right = 1	1.52%	
	Left = 1	1.52%	
Normal pressure 16	Bilateral = 8	12.12%	24.24%
	Right = 4	6.06%	
	Left = 4	6.06%	

Comparative study of the effects of enlarged adenoids on middle ear pressure and hearing is shown in Table VIII.

Table VIII: Correlation between pressure change and hearing loss (n-92)

Only pressure change (n-66)	Only hearing loss (n-92)	Both pressure change and hearing loss	Total number of ears affected	Normal
50 (75.76%)	76 (82.61%)	76 (82.61%)	76 (82.61%)	16 (17.39%)

Discussion

In this study, enlarged adenoid was found in 1.15% children under 15 years of age. The patients were collected from hospital of national referral level reflecting actual picture of the problem prevailing in the country. Majority of the patient presented with more than one symptom and the commonest symptoms was open mouth breathing, 34 (65.38%). Others symptoms included hearing impairment 27 (51.92%), snoring 23 (41.23%), nasal blockage and discharge 28 (40.38%). These mentioned findings were consistent with the findings of others reseachers.[1-4,7,15] Persistent discharge, epistaxis, cough and voice change, headache were found in 24.99%, 15.83%, 11.63% and 9.61% respectively. The symptoms were due to mechanical obstruction and associated secondary infections. Excessive salivation and dribbling of saliva 18 (34.61%) due to the fact that patient keep their mouth open to maintain airway. Excessive salivation and dribbling may occur due to persistent

exposure to environmental irritant. Sex incidence in this study were found 28 (53.80%) in male and 24 (46.20%) in female children. This incidence is similar to many other previous study.[1,3,16]

The highest incidence of 67.31% cases was between the ages of 6-10 years, which is inconsistent with the findings of others, where age incidence of enlarged adenoid found 3-6 years.[16] But this age incidence is quite consistent with many others who said adenoids were highest at the age of 6-8 years there after it regresses.[12] The late presentation is due to illiteracy, lack of knowledge about their problems and modern treatment and also receiving some traditional treatment for fear of operative procedure.

The present study revealed significant role of overcrowding, most of the children taking unbalanced diet are predisposing factors which causes enlarged adenoid due to recurrent upper respiratory tract infection. Incidence is higher among the children in a family having their number more than two. Behavioral study in this series showed stubborn nature due to deafness from OME, CSOM caused by enlarged adenoid.[7-9,20] Poor responses and performance at classes may be due to reduced mental alertness[16] and impaired hearing[18] caused by enlarged adenoid which is consistent with other.[4]

Examination of nasal cavity and paranasal sinus revealed no significant changes except nasal discharge possible from mechanical effects of enlarged adenoid and secondary infection. Also concomitant tonsillar enlargement was not observed indicating that their might be some factor other than immunological response causing preferential enlargement of adenoid.[14, 15]

Clinically, out of 104 ears 52 patients were examined by Pure-tone audiometry (PTA), and Impedance audiometry (Tympanometry) [5] were done in 92 ears (46 children) in present series. Conductive type of deafness was expected in 84.62% due to middle ear pathology. But it was found 47.74% by tuning fork test[6] (Rinne's test –ve, Weber's test lateralized to affected ears) & 82.61% by PTA, clinically otitis media with effusion (OME) was seen 59.62% but flat curve obtained by impedance audiometry in only 44 (66.66%) ears.

Inconsistency between clinical findings and test results may be due to facts that:

I. Non-cooperation due to inattention to test also poor intelligence.
II. Clinical examination done but test was not done in all cases.
III. Mild middle ear pathology like OME, retraction might not interfere middle ear compliance and hence hearing mechanism might remain unaffected.[19]

Ear findings in a significant 88 (84.62%) number in this study indicate an important

relationship between enlarged adenoid with middle ear pathology, of which OME 62 (59.62%) and CSOM 26 (24.99%) are common diseases. Reasonable percentage of CSOM in this series inconsistent with the findings of other authors.[11] In this series, comparative studies showed that the moderate to hugely enlarged adenoids involving ears mostly hearing loss 76 (82.61%) and pressure changes 50 (75.76%) in the middle ears. These changes mostly followed by right ear and least affected left ear. Bilateral involvement was due to extensive enlargement of adenoid tissue from midline interfering the tubal functions.[13] Right side was affected more than the left due to posture or disproportionate extension of adenoid behind right Eustachian tube.[20]

Negative middle ear pressure is due to enlarge adenoid causing mechanical obstruction to the eustachian tube resulting absorption of middle ear gases which is consistent with study of others.[2,10] Positive middle ear pressure was due to auto-inflation, excessive crying, swallowing, movement of soft palate and position the mouth which is similar to others.[17] Retraction of ear drum due to negative middle ear pressure commonly affecting bilaterally than unilateral. Total numbers of affected ear were 76 (82.61%).

Conclusion

Among the many childhood diseases, small percentage is diagnosed as having an enlarged adenoid with peak incidence between 6-10 years of age. Significant percentage has effect on middle ear pathology as OME, CSOM or permanent residual changes in the ear drum. In a few cases, orthodontic architectural deformity also develops resulting delayed speech development, impaired mental growth, physical and social complications. The morbidity can be prevented by primary health care education, early diagnosis and adequate treatment.

Acknowledgements

We express our gratitude to Director, Rajshahi Medical College Hospital for his kind co-operation to carry out our study. We also thanks to all staffs of ENT Department of Rajshahi Medical College Hospital for their cordial cooperation during this research work.

Contribution of the Authors

The first author was responsible for conception, design, acquisition of data analysis and interpretation of data for research. The second one assisted in compiling data and finalized results, computer composing and printing.

References

1. Ballantyne and John G. A synopsis of otolaryngology, 5th Edition 1987; p.343-345.
2. Bluestone CD, Cantekin EI. Eustachian Tube Dysfunction-in otology (Revised Ed.) Edited by G English Hegerstown MD. Harper and Raw Great Britain, Butter worth-Heinemann Ltd. 1979; p.1-40
3. Cowan DL. Logan Turner's Diseases of the Nose Throat and Ear. 10th ed. JF Broell, Great Britain, Butter worth-Heinemann Ltd. 1982; p.336-349.
4. Cohen D, Konak S. The evaluation of radiograph of the nasopharynx. Clin Otolaryngol. 1985;10: 73-78.
5. Cantekin EI. Tympanometric pattern of classification in relation to middle ear effusion. The Laryngoscope. 1975;84: 56-64.
6. Maw AR, Capper JW, Slack. Tuning fork test in children – an evaluation of their usefulness. J Laryngol Otol. 1987;102(8): 780-783.
7. Deweese DD, Saunders WII. Text book of otolaryngology. 6th ed, St. Louis The C.V Mosby company: 1982,67;p. 386.
8. Draper WL. Secrotory otitis media in children. Laryngoscope, 1967;77, p. 616-633.
9. Ervin J, Ostfeld MD. Transient Pressure Changes in the middle ear. Arch Otolaryngeal head neck surgeon, 1991;117(12): 1390-1394.
10. Fujiaka M, Young LW, Girday BR. Radiographic evaluation of adenoidal size in children: Adenoidal-Nasopharyngeal ratio AJR 1997;133: 401-404.
11. Gates, Harris, Avery. Predictive value of Tympanometric in middle ear effusion – American Otological S Meami Beach Florida. 1985; 5: 25-26
12. Hibbert J, Stell PM. The role of enlarged Adenoid in the etiology of serous otitis media. Clin Otolaryngol. 1982;7: 253-256.
13. Hibbert J, Stell PM, Write A. Value of Physical Signs in the diagnosis of enlarged adenoids, Clin Otolaryngol. 1980;5: 191-194.
14. Hibbert J, Tweedie MCK. The value of signs and symptoms in the diagnosis of enlarged adenoids, Clin Otolaryngol. 1977;2: 293.
15. Mawson SR. Scolt – Brawn's diseases of ear nose throat, 6th ed, Jorden Hill, Oxford Great Britain, Butter worth-Heinemann Ltd, 1979; 5 p. 257-259
16. Maw AR, Jeans DOD, Fernando DCS. Inter observer variability in the clinical and radiological assessment with adenoid Volume–Clin Otolaryngol. 1981;6: 317-322.
17. Mawsion SR, Fagan P. Tympanic effusion in children. J Laryngol Otol. 1972;86: 105-119.
18. Palva T. Clinical otolaryngology. 1st ed. AGD Maran and PM Stell, 1979;p.499.
19. Staloff J, Menduke H. Adenoid and Hearing Loss in Children. Am J Dis-Child, 1958;95: 529-533.
20. Tumerkin A. Pre-Epidermosis. J Laryngol. 1961;75: 487.

North Bengal Medical College Journal
Vol 2 No 2 July 2016

Original Article

Spectrum of Fine Needle Aspiration Cytology in palpable Breast Lumps

Shaheen Akter,[1] Md Jahidul Islam,[2] Md Shariful Haque[3]

Revised :October 20, 2015 Accepted :December 04, 2015

Abstract

Introduction: *Fine Needle Aspiration Cytology (FNAC) is a well-recognized method of investigation in breast lumps and has high sensitivity and specificity. FNAC is being performed as a pre-operative test to evaluate breast lumps and can prevent unnecessary major surgery. In this study, the spectrum and frequency of FNAC diagnoses in breast lumps and also the accuracy of the needle tip localizing the tumor during FNAC procedure were assessed.*

Methods: *It was a hospital based cross-sectional study conducted in the Pathology department of North Bengal Medical College. All FNAC of female breast lumps reported between January 2013 to December 2015 were included in this study. The age of the patient, size of the lump, frequency and percentage of different types of cytology diagnosis were assessed.*

Results: *A total of 590 FNAC cases were reported. Age ranges from 16-84 years with a mean age of 41.68 years. Among the lesions, 208 (35.25%) fibroadenoma, 123 (20.85%) fibrocystic disease, 113 (19.15%) carcinoma, 66 (11.19%) abscess, 05 (0.85%) chronic mastitis and 14(2.37%) cystic lesion were identified. Some 68.58% cases of fibroadenoma and 46.01% cases of carcinoma were in the age group 21-30 years and 41-50 years respectively. Since inadequate sample on aspiration is 2.03% in our study, the accuracy rate of needle tip in localizing the tumor in fine needle aspiration cytology is 97.97%.*

Conclusion: *FNAC is one of the tools for breast lump diagnosis which needs experienced hands high specificity. Fibroadenoma was the commonest lesion in this study followed by fibrocystic disease. Malignancy was detected as the third commonest lesion.*

Key words: *FNAC; Breast lump, Fibroadenoma*

North Bengal Med. Coll.J. 2016; 2 (2) : 23-32

1. *Associate Professor of Pathology, North Bengal Medical College, Sirajganj*
2. *Assistant Professor of Surgery, Shaheed M. Monsur Ali Medical College, Sirajganj*
3. *Assistant Professor of Nephrology, Shaheed M. Monsur Ali Medical College, Sirajganj*

Correspondence *Shaheen Akter, Email: drjahidul@gmail.com*

Introduction

Palpable breast lump is a common diagnostic problem to both general practitioners and surgeons. About 10% women use to visit hospital with breast lumps as the chief complaint. 80-85% of breast lumps are benign and rests are malignant.[1,2,3] Breast carcinoma is the leading cause of cancer incidence and death in women. It is now the most common cancer both in developed and developing countries.[4] Early detection is the mainstay in management of breast carcinoma.

Excisional biopsy was accepted practice in the past, but presently needle biopsy makes it possible to reduce surgical excision of benign breast lesions to a minimum. The main purpose of fine needle aspiration cytology (FNAC) of breast lumps is to confirm cancer pre-operatively and to avoid major surgery in specific benign conditions.[5] Breast carcinoma is the most common malignant neoplasm and the leading cause of death from cancer in women, with more than one million cases occurring worldwide annually.[6] A large number of patients in Bangladesh have been suffering from breast cancer. Each year the number of patient is increasing. Because of existing social circumstances, female patients are hesitant to be examined by the clinicians for breast lump and the patients report it in advanced stage of malignancy.

FNAC could provide a diagnosis with only 10-30% of the cost of surgical biopsy. Ninety five percent accuracy in preoperative diagnosis of mammary cancer by clinico-cytological combination was reported in a study.[7] As FNAC became more reliable in diagnosing malignancy and thereby the use of frozen section histology had been reduced by about 80 %.[8] Now-a-days, FNAC is being performed as a pre-operative test to evaluate the breast lump. The result of FNAC in palpable breast lumps showed a very high sensitivity, specificity and accuracy. FNAC can prevent unnecessary surgery also. The present study is intended to look the frequency distribution of different lesions in FNAC of palpable breast lumps.

Materials and Methods

It is a hospital based cross-sectional study carried out from the data retrieved from the Department of Pathology of North Bengal Medical College Sirajganj, Bangladesh in a period from January 2013 to December 2015. 590 palpable breast lumps underwent fine needle aspiration cytology (FNAC) in this period. All FNAC patients were female.

Clinical Methods: FNAC were performed with 21 gz needle and 10 cc syringe. Aspiration slides were routinely stained with PAP stain. All the FNAC cases have been analyzed according to the age of the patient, size of the lump, frequency and percentage of different types of cytology diagnosis.

Results

The age distribution of the patients shown in (Table I). The age incidence was ranged from 16 to 84 years (mean age 41.68 years). The age incidence for the benign lesions ranged from 16 years to 51 years (means age 30.89 years). The incidence for the malignant lesions ranged from 25 to 84 years (mean age 47.25 years). The incidence for the inflammatory lesions ranged from 23 to 47 years (mean age 33.25 years). The incidence for the cystic lesions ranged from 32 to 54 years (mean age 42.20 years). The most common age group for benign lesions was between 21 to 30 years and for the malignant lesion was 41 to 50 years. All the patients complained of lump in the breast. The other symptoms were pain in the lump, discharge per nipple and lump in the axilla. The duration of symptoms varied from few weeks to few year.

Table I: Distribution of all FNAC cases according to age groups (n=590)

Age in years	Inflammat-ory n (%)	Cystic lesion n (%)	Benign n (%)	Suspicious carcinoma n (%)	Carcinoma n (%)	Inadequate sample n (%)	Total
11-20	0 (0%)	0 (0%)	50 (8.47%)	0 (0%)	0 (0%)	0 (0%)	50 (8.47%)
21-30	32 (5.42%)	7(1.19%)	179 (30.34%)	0 (0%)	5 (0.85%)	7 (1.19%)	230 (38.98%)
31-40	37(6.27%)	4(0.68)	128(21.69%)	1 (0.17%)	16 (2.71%)	5 (0.85%)	191(32.37%)
41-50	5 (0.85%)	2(0.34%)	14 (2.37%)	1 (0.17%)	52 (8.81%)	0 (0%)	74 (12.54%)
51-60	0 (0%)	1(0.17%)	2 (0.34%)	1 (0.17%)	20 (3.39%)	0 (0%)	24 (4.07%)
61-70	0 (0%)	0 (0%)	0 (0%)	1 (0.17%)	17 (2.28%)	0 (0%)	18 (3.05%)
>71	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (0.51%)	0 (0%)	3(0.51%)
Total	**74 (12.5%)**	**14 (2.37%)**	**373 (63.22%)**	**4 (0.68%)**	**113 (19.1%)**	**12 (2.03%)**	**590 (100%)**

All the different FNAC diagnoses with their respective frequencies have been shown in (Table II). Majority of the cases, 373 (63.22%) were found to be benign (Figure-I) in contrast to 117 (19.83%) reported either as suspicious of carcinoma or as carcinoma (Figure-II). Among the types of the lesions, fibroadenoma showed the highest incidence 208 (35.25%) followed by fibrocystic disease 123 (20.85%) and carcinoma 113 (19.15%) with suspicious of carcinoma in 4 cases. Inflammatory lesions were abscess 66 (11.19%), chronic mastitis 05 (0.85%), and fat necrosis 03 (0.51%). Cystic lesions were 14 cases (2.37%) and commonest was galactocele in 10 cases. Some 12 (2.03%) cases were designated as ‘others’ included fatty tissue, unsatisfactory smears. Among

malignant lesions 71 (60.68%) were presented with a size less than 2 cm and 43 (36.75%) presented with 2-5 cm.

Table II: Frequency distribution of different categories of Lesions

Lesion catagory	Diagnosis	Number of FNAC cases (%)
Inflamatory n= 74	Abscess	66 (11.19%)
	Choronic mastitis	5 (0.85%)
	Fat necrosis	3 (0.51%)
Cystic lesion n= 14	Galactocele	10 (1.69%)
	Benign cystic lesion	4 (0.68%)
Benign neoplasm n= 373	Fibroadenoma	208 (35.25%)
	Fibrocystic change	123 (20.85%)
	Proliferative breast disease	42(7.12%)
Malignant neoplasm n=117	Suspicious for carcinoma	4 (0.68%)
	Carcinoma	113 (19.1%)
Other n=12	Inadequate sample	12 (2.03%)
Total		**590 (100%)**

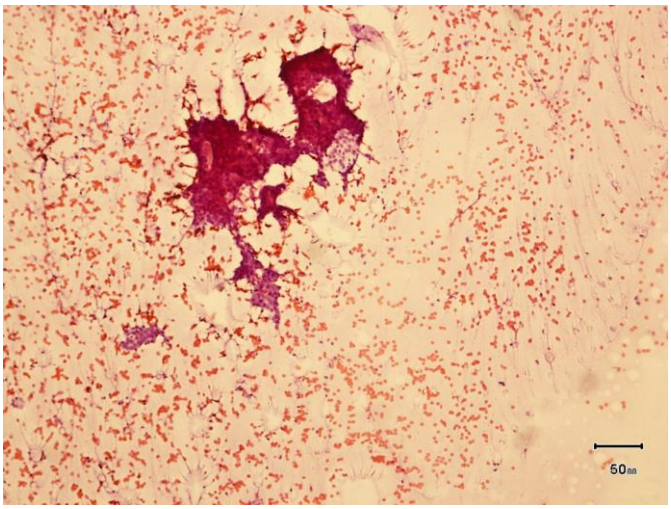

Figure I: FNAC showing clusters of benign looking ductal epithelial cells-diagnosed as "Fibroadenoma", pap stain, 100x

In cytology, twelve cases among the 590 fine needle aspiration cytology were reported as inadequate sampling (unsatisfactory) based on the presence of normal glandular cells. On repeat fine needle aspiration with ultrasound guidance,

all were reported as fibroadenoma. Patient underwent local excision of the tumor and histopathological confirmation later. Thus inadequate sampling rate was 2.03%. Accuracy rate of the needle tip in localizing the tumor in fine needle aspiration cytology was 97.97%.

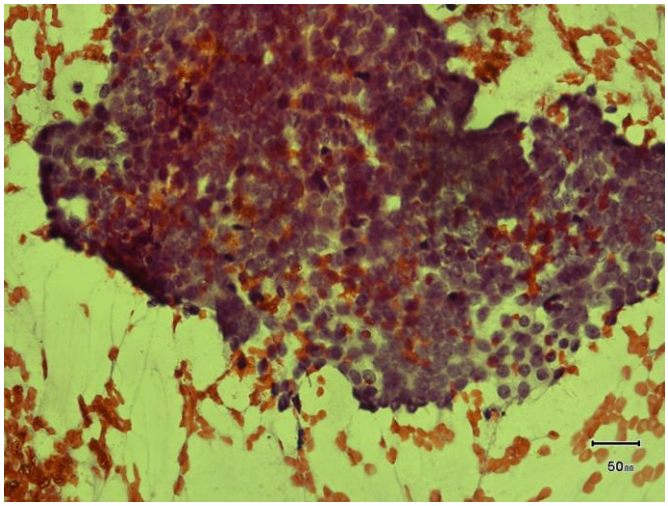

Figure II: FNAC showing clusters of malignant cells with hyperchromatic nuclei and prominent nucleoli – diagnosed as "Carcinoma", pap stain, 400x

Discussion

Breast is an important and popular site for fine needle aspiration cytology. In 1984, Wanebo et al[1] suggested fine needle aspiration in place of open surgical biopsy for the diagnosis of breast cancer. There is an increasing tendency to confirm the diagnosis of the breast cancer at first consultation by some form of needle biopsy technique. This allows better investigation and wiser preoperative discussion than was possible when excision biopsy and frozen section confirmed the clinical diagnosis. The breast lump is usually discovered by the patient. In premenopausal women, up to 80% are benign; where as in patients over the age of 60 approximately 90% of the breast lumps are malignant. The fine needle aspiration cytology has become the investigation of choice for the diagnosis of the breast malignancy. The typical carcinoma presents a gritty resistance to the fine needle. The aspirate is usually copious and blood stained. [9]

A total of 74 (12.54%) cases of inflammatory lesions of the breast were found in this study. This result are supported by the findings from Pakistan 15% by Bukhari et al. [10] Results showed little higher from Nepal 22.6% by Kumar[11] and findings of Nemaqani and Yaqoob[12] showed (26.5%) from Kingdom of Saudi Arabia.

We also detected 10 (1.69%) cases of galactocele, which revealed milk during aspiration and microscopically histiocyte in the background of milk. Majority of the

cases were in the age group of 21-30 yrs which is the most active reproductive years.

In this study we had 373 benign lesions (63.22%) in FNAC, fibroadenoma being the most common benign lesion that presents for needle aspiration. This has been confirmed in other series also. Fibroadenoma was found (35.25%) cause of the breast lump in this study and 68.58% were in the age group of 21-30 years. This frequency of fibroadenoma was near similar (38.3%), to the findings of Rahman et al.[13] From Sudan among their 200 cases Ahmed et al.[14] found fibroadenoma in (28%) of cases. The findings of Kumar[11] and Mayun et al.[15] as their result were 22% and 23.7%. Besides Bukhari et al.[10] showed 16% and Pradhan and Dhakal[16] showed only 8% of fibroadenoma cases. The higher rate than the last studies was perhaps caused by increased awareness among young women about the breast lump in this country. Mayun et al.[15] found average age of fibroadenoma was 16 years but this study found most of the cases in the age group of 21-30 years. This demands further investigation to find out whether there is any cause of this age difference in relation to inhabitation or ethnicity.

In this study, 117 (19.83%) malignant cases were detected. Previous study in Bangladesh by Rahman et al.[17] showed 14.17% malignancy and Rupom et al.[18] showed 13.74% of malignant cases in FNAC. Pradhan and Dhakal [16] also reported 15.5% malignant cases among their 2246 cases. Though Yip et al.[19] and Bukhari and Akhter[20] found near results but their numbers of cases were relatively smaller, 676 and 175 respectively. Ahmed et al.[14] in Sudan, Mayun et al.[15] in Nigeria and Bukhari et al.[10] in Pakistan reported 30.5%, 40% and 31% malignant cases. A study done by Khatun et al.[21] showed only 14/310 (4.32%) of malignant cases. 41-50 age group showed highest number of malignant cases 52 (46.01%) and we may conclude the majority (60.17%) of the patients found in the middle age from 31-50 years. Majority of the patients (65.8%) in the age group of 31- 50 years were also observed by Sandhu et al.[22] in India and Rupom et al.[18] found highest requency in the 4th decade of life.

However, reports from the western world show that female breast carcinoma is predominantly seen in the fifth and sixth decades.[23-25] Farooq and Coleman[26] compare age incidence between the South Asian and Non- South Asian breast carcinoma patient in England and Wales and found mean age at diagnosis of the South Asian women were 51.8 years compared with 62.8 years for non-south Asians and 16% (compared with 5%) aged under 40 years at diagnosis.

The study finding showed similarity in age of carcinoma patients with that of Sandhu et al.[22] It showed that mean age of our female breast cancer patients was found to be lower compared to the western world with an average difference of one decade. Early age of menopause in Indian females in comparison to their western counterparts has

been observed in the past.[27] The earlier published reports also show that the risk of breast carcinoma increases with increasing age of menopause, possibly because the women are exposed to hormones for a longer duration. [28-30]

In another prospective study, it was concluded that fine needle aspiration cytology and core needle biopsy is complementary in the accurate diagnosis of the breast cancer.[31] Fine needle aspiration cytology compliments clinical and radiological diagnosis; thus triple assessment has been reported to produce 99% accuracy for benign and malignant lesions. The diagnostic accuracy of clinical examination, mammography and fine needle aspiration cytology was compared with the definitive histological findings. Comparative study of all 3 diagnostic techniques in the diagnosis of breast tumor has shown that the accuracy of 99% can be achieved.[32]

The accuracy of the needle tip in localizing the tumor in fine needle aspiration cytology was also studied in our series by comparing the normal glandular cell aspirate and inadequate aspirate with the tumor cell aspirate. The unsatisfactory (inadequate) sampling in which there was little or no cellular material reported, we believe, to be an error in the technique of aspiration. In our study, we had twelve aspirations which were reported as unsatisfactory, bringing the inadequate sampling rate to 2.03%. The proportion of inadequate sampling as reported by different studies varies from 9 to 18%.[33] The accuracy rate of needle tip in localizing the tumor in fine needle aspiration cytology in present study was 97.97%. Repeat fine needle aspiration under ultrasound guidance was performed on the inadequate sampling specimen and that time it was reported as fibrodinoma. By different studies it has been also concluded that accuracy of fine needle aspiration cytology in diagnosing the breast tumors increases by performing repeat aspiration under ultrasound guidance in a lump for which previously been reported as inadequate sampling.[33]

Apart from the high accuracy rate of fine needle aspiration cytology, some have raised questions about the possible dangers of cell implantation from the needle aspiration. These rare reports have largely resulted from the use of larger cutting needle (18 gauze) rather than fine needles (21 gauze). With this fine needle technique, there is essentially no danger of implantation with breast aspiration.[34] Franzen and Zajicek in a review of 3479 consecutive breast aspirates found no evidence of seeding along the needle tract.[35] This is not surprising as the needle tract is invariably removed with definitive surgery.

Conclusion

Fibroadenoma was the commonest lesion found in this study and was found mostly in the age group of 21-30 years, second commonest was fibrocystic disease.

Malignancy was detected as the third common lesion and majority was found in 41-50 years age group. Breast carcinoma patients of this region were at the lower age of one decade than that of western women. FNAC is commonly used as a part of diagnostic triad in case of breast lump, which in addition to clinical breast examination and imaging. This technique is quite attractive because of its rapidity of execution and interpretation, its low cost (compare to open biopsy of any type), and its low rate of morbidity.

Contribution of the Authors

The first author was responsible for collection of samples, interpretation of data. Others helped for computer composing and data analysis.

References

1. William HH, Pamela AP, Elaine YP. The use of fine needle aspiration in the evaluation of persistent palpable dominant breast masses. Am J Obstet Gynecol. 1993; 168: 1815-1819.
2. Johnson. Breast fine needle aspiration cytology and core biopsy: a guide for practice. Cancer. 2003;19: 203-208.
3. Ariga R, Bloom K, Vijaya B Reddy VB. Fine needle aspiration of clinically suspicious palpable breast mass with histopathological correlation. Am J Surg. 2002; 184: 410-413.
4. Rubin M, Horiuchi K, Joy N. Use of fine needle aspiration for solid breast lesion is accurate and cost effective. Am J Surg. 1997; 174: 694-698.
5. Yalavarthi S, Tanikella R, Prabhala S, Tallam US. Histopathological and cytological correlation of tumors of breast. Med J D Y Patil Univ. 2014;7: 326-331.
6. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. Int J Cancer. 2001; 94: 153-156.
7. Russ JE, Winchester DP, Scanlon EF, Christ MA. Cytologic findings of aspiration of tumors of the breast. Surg Gynecol Obstet. 1978; 146: 407-411.
8. Gardecki TI, Hogbin BM, Melcher DH, Smith RS. Aspiration cytology in the preoperative management of breast cancer. Lancet. 1980; 2: 790-792.
9. Hebbar AK, Iyanna H. Prospective study of fine needle aspiration cytology of clinically palpable breast lump with histopathological correlation. Int J Res Med Sci. 2013;1: 257-262.
10. Bukhari MH, Arshad M, Jamal S, Niazi S, Bashir S. Use of fine needle aspiration in the evaluation of breast lumps. Patholog Res Int. 2011: 689521.
11. Kumar R. A clinicopathologic study of breast lumps in Bhairahwa, Nepal. Asian Pac J Cancer Prev. 2010; 11: 855-858.
12. Nemenqani D, Yaqoob N. Fine needle aspiration cytology of inflammatory breast lesions. J Pak Med Assoc. 2009; 59: 167-170.

13. Rahman MZ, Sikder AM, Nabi SR. Diagnosis of breast lump by fine needle aspiration cytology and mammography. Mymensingh Med. J 2011; 20: 658-664.
14. Ahmed HG, Ali AS, Almobarak AO. Utility of fine-needle aspiration as a diagnostic technique in breast lumps. Diagn Cytopathol. 2009; 37: 881-884.
15. Mayun AA, Pindiga UH, Babayo UD. Pattern of histopathological diagnosis of breast lesions in Gombe, Nigeria. Niger J Med. 2008; 17: 159-162.
16. Pradhan M, Dhakal HP. Study of breast lump of 2246 cases by fine needle aspiration. JNMA J Nepal Med Assoc. 2008; 47: 205-209.
17. Rahman MZ, Islam S. Fine Needle Aspiration Cytology of Palpable Breast Lump: A Study of 1778 Cases. Surg Curr Res. 2013; S12: 001.
18. Rupom TU, Choudhury T, Banu SG. Study of Fine Needle Aspiration Cytology of Breast Lump: Correlation of Cytologically Malignant Cases with Their Histological Findings. BSMMU J. 2011; 4: 60-64.
19. Yip CH, Jayaram G, Alhady SF. The experience with fine needle aspiration cytology in the management of palpable breast lumps in the University Hospital Kuala Lumpur. Med J Malaysia. 2000; 55: 363-367.
20. Bukhari MH, Akhtar ZM. Comparison of accuracy of diagnostic modalities for evaluation of breast cancer with review of literature. Diagn Cytopathol. 2009; 37:416-424.
21. Khatun H, Tareak-Al-Nasir, Enam S, Hussain M, Begum M. Correlation of fine needle aspiration cytology and its histopathology in diagnosis of breast lumps. Bangladesh Med Res Counc Bull. 2002; 28: 77-81.
22. Sandhu DS, Sandhu S, Karwasra RK, Marwah S. Profile of breast cancer patients at a tertiary care hospital in north India. Indian J Cancer. 2010; 47: 16-22.
23. Yeoh GP, Chan KW. Fine needle aspiration of breast masses: an analysis of 1533 cases in private practice. Hong Kong Med J. 1998; 4: 283-288.
24. Anderson WF, Chatterjee N, Ershler WB, Brawley OW. Estrogen receptor breast cancer phenotypes in the Surveillance, Epidemiology, and End Results database. Breast Cancer Res Treat. 2002; 76: 27-36.
25. El-Tamer MB, Wait RB. Age at presentation of African-American and Caucasian breast cancer patients. J Am Coll Surg. 1999; 188: 237-240.
26. Farooq S, Coleman MP. Breast cancer survival in South Asian women in England and Wales. J Epidemiol Community Health. 2005; 59: 402-406.
27. Bharadwaj JA, Kendurkar SM, Vaidya PR. Age and symptomatology of menopause in Indian women. J Postgrad Med. 1983;29: 218-222.
28. McPherson K, Steel CM, Dixon JM. ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. BMJ. 2000; 321: 624-628.

29. Alberg AJ, Lam AP, Helzlsouer KJ. Epidemiology, prevention, and early detection of breast cancer. Curr Opin Oncol. 2000; 11: 435-441.
30. Trichopoulos D, MacMahon B, Cole P. Menopause and breast cancer risk. J Natl Cancer Inst. 1972; 48: 605-613.
31. Dennison. A Prospective Study of the Use of Fine Needle Aspiration Cytology and Core Biopsy in the Diagnosis of Breast Cancer. Br J Surg. 2003; 9:491-497.
32. Dixon, TJ, Anderson J, Lamb SJ, Nixon APM, Forrest. Fine needle aspiration cytology in relationship to clinical examination and mammography in the diagnosis of a solid breast mass. Br J Surg. 1978; 71:593-596.
33. Saxe A, Phillips E, Orfanou P, Husain M. Role of sample adequacy in fine needle aspiration biopsy of palpable breast lesions. Am J Surg. 2001; 182:369-371.
34. Shabot M, Goldberg IM, Schick P, Nieberg R Pilchy H. Aspiration cytology is superior to tru-cut needle biopsy in establishing the diagnosis of clinically suspicious breast masses. Ann Surg. 1982; 196:122-126.
35. Franzen S, Zajoak J. Aspiration biopsy in diagnosis of palpable lesions of the breast. Acta Radiol. 1968; 7: 241-262.

North Bengal Medical College Journal
Vol 2 No 2 July 2016

Original Article

Association of Serum Homocysteine Concentration in Patients with Acute Coronary Syndrome

Md. Samshul Alom,[1] Md. Zillur Rahman,[2] Mohammad Azizur Rahman,[3] Abdul Wadud Chowdhury [4]

Revised : January 28, 2016 Accepted : March 05, 2016

Abstract

Introduction: *Coronary artery disease (CAD) has become the most common cause of mortality and morbidity in the entire world. The aim of the study was to find out the association between serum homocysteine level and acute coronary syndrome (ACS).*

Methods: *This was a case control study, conducted in the department of Cardiology, Dhaka Medical College Hospital, Dhaka, during the period of July 2011 to December 2011. In this period, newly diagnosed patients with ACS were taken as cases, and age, sex matched healthy subjects with normal ECG were taken as controls.*

Results: *Total 120 cases were studied. Serum homocysteine level ≤I5 µmol/L was found in 28 (46.7%) cases without CAD and risk factor and in 52 (86.7%) controls. Serum homocysteine level >15 µmol/L was found in 32 (53.3%) caswes with CAD whereas 8 (13.3%) in controls.*

Conclusion: *Serum total homocysteine concentration (tHcy) is recognized as an independent and important risk factor for ACS patients. As a result, we should to plan strategies for reduction of serum homocysteine concentration in both the ACS patients and the high risk population.*

Key words: *Homocysteine, Acute coronary syndrome. High risk population*

North Bengal Med. Coll.J. 2016; 2 (2) : 33-41

1. *Assistant professor, Department of Cardiology, North Bengal Medical College, Sirajganj*
2. *Assistant professor, Department of Medicine, North Bengal Medical College, Sirajganj*
3. *Associate professor, Department of Respiratory Medicine, North Bengal Medical College, Sirajganj*
4. *Professor and Head, Department of Cardiology, Dhaka Medical College, Dhaka*

Correspondence *Md. Shamshul Alom,Email:dr.swapannbmch@gmail.com*

Introduction

Coronary artery disease (CAD) has become a major health problem and is the most common cause of mortality & morbidity in the entire world.[1] Among the coronary artery diseases, acute coronary syndrome is the leading cause of death in the developed countries & second leading cause of death in developing countries. It has been estimated that, by the year 2020, coronary artery disease (CAD) will hold first place in the WHO's list of leading cause of disability.[2] About 7.1 million deaths occurred globally in 1999 due to CAD and it will rise to 11.1 million by 2020. In the United Kingdom (UK), 1.3 million people develops CAD every year while in USA, 0.8 million people suffers from new heart attacks each year. In India 4% rural and 11% urban population suffers from CAD. The progressively increasing trend of the disease in our country shows that the prevalence was 3.3/1000 in 1976 and 17.2/1000 in 1986 indicating a 5 fold increase in 10 years.[4]

Acute coronary syndrome (ACS) constitutes a spectrum of clinical presentations, ranging from unstable angina (UA) through non-ST segment elevation myocardial infarction (NSTEMI) to ST segment elevation myocardial infarction (STEMI). It is a multifactoral disease involving well-known risk-factors such as age, male sex, smoking, hypertension (HTN), diabetes mellitus (DM), obesity, hypercholesterolemia, family history of premature CAD & sedentary lifestyle.[3] An ACS develops when a vulnerable or high risk atheromatous plaque (having a thin fibrous cap and a large lipid core with abundant inflammatory cells accumulation) undergoes disruption of its fibrous cap. Following plaque-rupture, a sufficient quantity of thrombogenic substances are exposed, and the coronary artery lumen may become partially or completely occluded by a combination of platelet-aggregates, fibrin and red blood cells. Completely occlusive thrombus leads to STEMI, while sub-totally occlusive thrombus leads to NSTEMI or UA.[4]

There is a growing recognition that high level of serum homocysteine is associated with atherosclerotic vascular diseases including CAD. This started in the late 1960s when McCully KS et al.[5] a pathologist in Boston, encountered two children with elevated serum homocysteine concentrations and homocystinuria, who, despite being very young, had autopsy evidence of extensive arterial thrombosis and atherosclerosis. Several studies have also demonstrated that the presence of moderate hyperhomocysteinemia is an independent risk factor for atherosclerosis in the coronary, cerebral and peripheral vasculature.[6,7,8]

Several studies have attempted to establish the prevalence of hyperhomocysteinemia in patients with accelerated vascular diseases. In general, case control studies have been robust in confirming the association

between hyperhomocysteinemia and coronary artery disease (CAD). In a meta-analysis, Boushey CJ et al.[9] estimated that an increase of 5 µmol/L in the serum total homocysteine concentration (tHcy) raises the risk of coronary artery disease by as much as an increase of 20 mg/dl (0.52 mmol/L) in the serum cholesterol concentration.

Though, several studies have been undertaken in the past in India and abroad, regarding the association of high serum homocysteine concentration as a risk factor for ACS, this association has not been studied in our country. That's why, we designed this study to evaluate the association of high serum homocystine concentration which can play an important role as a risk factor for ACS.

Materials and Methods

This was a case-control study which was conducted in the Department of Cardiology, Dhaka Medical College and Hospital, Dhaka, during the period of July 2011 to December 2011. Study population was all the patients with Acute Coronary Syndrome (ACS) within the study period and healthy population within that period. Newly diagnosed patients with ACS admitted in the Coronary Care Unit (CCU) of Dhaka Medical College Hospital, Dhaka, were taken as cases and age & sex matched healthy subjects (doctors, medical students, nurses and other hospital-staffs and patient attendants' from DMCH, Dhaka, with no history of ischaemic heart disease (IHD) and normal ECG was taken as control. In this study, total number of sample was 120, among which cases (patient) 60 and control (normal) were 60.

Selection Criteria:

Inclusion criteria for cases :

Newly diagnosed Acute Coronary Syndrome (ACS) patients having characteristic ischaemic type chest pain with characteristic ECG and cardiac bio-marker (Troponin I) findings of ACS. Those were presenting for the first time in the Coronary Care Unit (CCU) of DMCH, Dhaka is also included.

Inclusion criteria for control:

Age and sex matched healthy subjects having no history of ischaemic heart disease (IHD) and normal ECG

Exclusion criteria:

Study subjects having previous history of myocardial infarction / unstable angina / Percutaneous Coronary Intervention (PCI) / Coronary Artery Bypass Graft Surgery (CABG). Those subjects having cardiomyopathy, congenital heart disease or valvular heart disease and were unwilling to be included in the study, already getting folic acid, vit. B_6 or vit. B_{12} supplementation are excluded.

Data collection procedure: Data were collected by using a preformed data sheet in the following manner:

Informed consent was taken from all cases & controls or from their legal guardians. Initial evaluation of the study population by name, age, sex, height, weight, clinical history and examination were performed and recorded accordingly. Risk factors of coronary artery disease (CAD) like hypertension (HTN), smoking, dyslipida-emia diabetes mellitus (DM), family history of premature CAD and obesity were noted from all cases and controls. Fasting blood samples were collected for serum homocysteine assay on the morning following the admission day from the cases & fasting morning- samples were collected from the controls. Serum homocysteine level was measured by Fluorescence Polarization Immunoassay (FPIA) method and recorded in units of μmol/L from Biochemistry Department of Bangabandhu Sheikh Mujib Medical University. Level of serum homocysteine was grouped as follows:

- Normal homocysteine level: ≤I5 μmol/L.
- High homocysteine level: >15 μmol/L.

Results

After collection, data were checked for consistency, before entry in the SPSS and analysis, the results were presented in tables. The description highlights the main feature. P value calculated using unpaired 't' test.

ns = not significant

The study included 120 subjects and the mean age of the total study subjects was 57.89±38.90 years ranging from 21 to 80 years. Among the cases, the mean age was 57.7±38.80 years and among the controls, the mean age was 58.33±37.79 years. Maximum number was found in the age group of 36-50 years in both cases and controls. P value calculated using chi square test (Table 1)

Table I: Age Distribution of the Study Subjects (n-120)

Age in years	Case (n-60)		Control (n-60)		Total (n-120)		P value
	n	%	n	%	n	%	
21-35	12	20.0	9	15.0	21	17.5	
36-50	23	38.3	25	41.7	48	40.0	
51-65	22	36.7	24	40.0	46	38.3	
66-80	3	5.0	2	3.3	5	4.2	
Mean±SD	57.7±38.80		58.33±37.79		57.89±38.90		0.761^{ns}
Range (min-max)	(21-80)		(21-80)		(21-80)		

Table II: Sex distribution of the study subjects (n-120)

Sex	Case (n-60)		Control (n-60)		Total (n-120)		P value
	n	%	n	%	n	%	
Male	52	86.7	51	85.0	103	85.8	
Female	8	13.3	9	15.0	17	14.2	0.793[ns]

ns = not significant

Among the 120 subjects, 52(86.7%) and 51(85.0%) were male in the case and control respectively. Females were found 8(13.3%) in the cases and 9 (15.0%) in the controls. Male-female ratio was 6:1 in the whole study subjects. The results are shown in the Table II.

Table III: Distribution of the study subjects according to Serum Homocysteine level (μmol/L) (n-120)

S. Homocysteine level (μmol/L)	Case (n-60)		Control (n-60)		Total (n-120)		P Value
	n	%	n	%	n	%	
≤15	28	46.7	52	86.7	80	66.7	0.001[s]
>15	32	53.3	8	13.3	40	33.3	
Mean±SD	19.5±16.3		12.6±3.3		19.5±12.1		0.002[s]
Range (min - max)	(7.3-129.0)		(7.1-31.4)		(7.1-129.0)		

s= significant, p value calculated from Chi square test and from unpaired 't' test

Serum Homocysteine level ≤15 μmol/L was found in 28 (46.7%) cases and in 52 (86.7%) controls. Serum Homocysteine level > 15 μmol/L was found in 32 (53.3%) cases and in 8 (13.3%) controls (Figure 1).

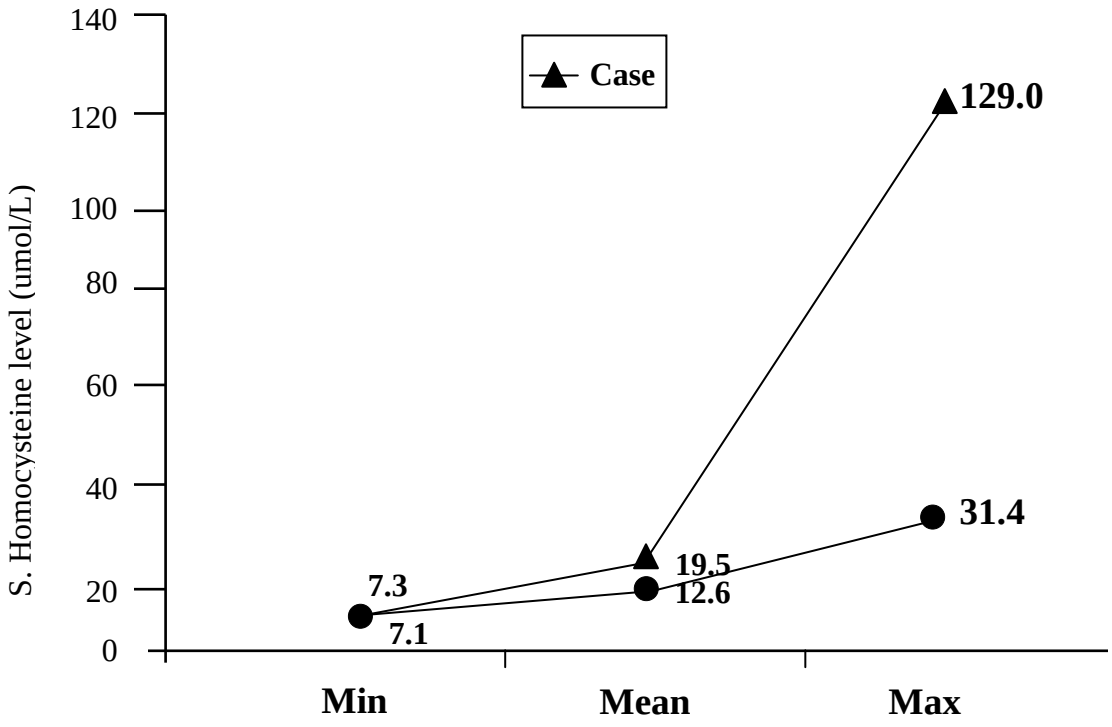

Figure 1: Line diagram showing mean, maximum and minimum distribution of Serum Homocysteine level (μmol/L) of the total study subjects (n-120)

Table IV: Risk-measurement of serum Homocysteine concentration for ACS patients (n-120)

Serum Homocysteine Level (μmol/L)	Case (n-60) [ACS present]		Control (n-60) [ACS absent]		Odds Ratio (OR)	95% CI for OR	P value
	n	%	n	%			
>15	32	53.3	8	13.3	7.43	2.80-20.34	0.001[s]
≤15	28	46.7	52	86.7			

s = significant, p value calculated from Chi square test

Serum Homocysteine level > 15 μmol/L was found in 32 (53.3%) cases and in 8 (13.3%) controls. Serum Homocysteine level ≤15 μmol/L was found in 28 (46.7%) cases and in 52 (86.7%) controls. The difference was statistically significant ($p<0.05$) in chi square test.

Table V: Distribution of the study subjects according to different risk factors for ACS (n-120)

Risk factors	Case (n-60)		Control (n-60)		P Value
	n	%	n	%	
Smoking	26	43.3	14	23.3	0.001[s]
Dyslipidaemia	33	55.0	13	21.7	0.001[s]
HTN	21	35.0	12	20.0	0.065[ns]
DM/IGT	14	23.3	7	11.7	0.093[ns]
F/H of premature CAD	14	23.3	8	13.3	0.609[ns]
Obese/Over-wt.	11	18.3	9	15.0	0.624[ns]

s = significant, ns= not significant, p value calculated from Chi square test.

Smoking, dyslipidaemia and HTN were more common risk factors in both cases and controls. However, Smoking and dyslipidaemia were significantly ($p<0.05$) higher in case group in Chi square test. Other risk factors like- HTN, DM/IGT, Family history (F/H) of premature CAD and Obese / Over-wt. were higher in number in cases than in controls

Table VI: Risk factors analysis for ACS (multiple logistic regression models) (n-120)

		95.0% CI for OR			
	OR	Lower	Upper	R^2	P value
HTN	2.71	0.98	7.47	0.17	0.054[ns]
DM/IGT	1.68	0.54	5.22	0.11	0.371[ns]
Smoking	4.19	1.61	10.85	0.41	0.003[s]
Obese/Over-wt.	1.29	0.44	3.79	0.09	0.646[ns]
Dyslipidaemia	5.94	2.16	16.35	0.35	0.001[s]
F/H of premature CAD	3.95	1.11	14.02	0.29	0.033[s]
S. Homocy steine level	6.70	2.48	18.06	0.44	0.001[s]
Constant	0.129				0.000

A smoker compared to a non smoker was 4.19 (95% CI 1.61 to 10.85) times more likely to have ACS. A dyslipidemic subject compared to a non dyslipidemic subject was 5.94 (95% CI 2.16 to 16.35) times more likely to have ACS. A subject with F/H of premature CAD was 3.95 (95% CI 1.11 to 14.02) times more likely to have ACS than those with no F/H of premature CAD. Smoking, dyslipidaemia, F/H of premature CAD and Serum Homocysteine level were found to be significantly ($p<0.05$) associated with ACS-risk; other risk factors (HTN, DM/IGT & obese/over-wt.) were not found to be significantly ($p<0.05$) associated with ACS-risk.

Discussion

This observational case-control study was carried out with an aim to find out the association between serum homocysteine concentration and acute coronary syndrome (ACS) and to assess the strength of association of serum homocysteine level with ACS patients as well as with the traditional risk factors of ACS. In this study, maximum number 48 (40.0%) was found in the age group of 36-50 years in both cases and controls. Males were predominant in this study and male female ratio was 6:1. In this study both cases & controls were divided into two sub groups according to serum homocysteine level which were ≤15 μmol/L and >15 μmol/L. Serum homocysteine level ≤15 μmol/L was found in 46.7% cases and in 86.7% controls. Serum homocysteine level >15 μmol/L was found in 53.3% cases and 13.3% in controls.

In our study it was observed that smoking (43.3% Vs 23.3%) and dyslipidaemia (55.0% Vs 21.7%) were significantly ($p<0.05$) higher in case group. However other risk factors like- HTN (35% vs. 20%), DM/IGT (23.3% vs. 11.7%), F/H of premature CAD (23.3% vs. 13.3%) and Obese/Over-wt. (18.3% vs. 15%) were higher in number in cases but not significantly ($p>0.05$) higher than controls. In Bangladesh, almost similar findings were also observed by Majumder et al.,[10] Siddique et al.[11] and Haque et al.[12]

In the present study, it was found that smoking (25.0% vs. 50.0%) and F/H of premature CAD (12.5% vs.30.0%) were significantly ($p<0.05$) higher in >15 μmol/L homocysteine level in the total study subjects. Other risk factors like HTN, DM/IGT, dyslipidaemia and obese/over wt. were not significantly ($p>0.05$) associated with high serum homocysteine level. Serum homocysteine level was significantly higher among the smokers, dyslipidaemics and those with family history of ischaemic heart disease ($p<0.05$), but no statistically significant association of serum homocysteine level was found with DM and HTN ($p>0.05$). The findings of this study were almost consistent with another study reported by Puri et al.[1]

Conclusion

This observational case-control study has conferred that, a high serum homocysteine concentration is an important & modifiable risk factor for acute coronary syndrome (ACS). As a result, although it was a single centre based study involving limited number of study-subjects, which may provide the basis of large future studies aimed at risk factor analysis in ACS patients as well as identification of the high risk population. The current study will also pave the way of planning strategies for reduction of serum homocysteine concentration in both the ACS patients and the high risk population by life-style modification, B-vitamins supplementation (Vit-B_6, Vit- B_{12} & Folic acid), food-fortification or newer methods in the coming days.

Contribution of the Authors

First author was the main researcher. Other authors helped in data collection, processing, statistical analysis and computer composing.

References

1. Puri A, Gupta OK, Dwivedi RN, Bharadwaj RPS, Narain VS, Singh S. Homocysteine and Lipid levels in young patients with coronary artery disease. JAPI. 2003;51: 681-685.
2. Ahmed M, Majumder AAS, Rahman A, Baqui MA. Relationship between baseline white blood cell count and angiographic severity of coronary artery disease in patients with acute coronary syndrome. Bangladesh Heart J. 2005;20(1): 6-10.
3. Gonzalez-Porras JR, Martin-Herrero F, Garcia-Sanz R, Lopez ML, Balanzategui A, Mateos MV, et al. Hyper-homocysteinemia is a risk factor of recurrent coronary event in young patients irrespective to the MTHFR C677T polymorphism. Thrombosis Research. 2007;119: 691-698.
4. Antman EM, Braunwald E. ST-elevation Myocardial Infarction: Pathology, Pathophysiology and clinical features, In: Libby P, Bonow RO, Mann DL, Zipes DP, Editors, Braunwald's Heart Disease. A text book of Cardiovascular Medicine, 8th edition, Saunders Elsevier, Philadelphia, USA, p. 1210.
5. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol. 1969;56: 111-128.
6. Boers GHJ, Smals AGH, Trijbels FJM, Fowler B, Bakkeren JA, Schoonderwaldt HC, et al. Heterozygosity for homocys-tinuria in premature peripheral and cerebral occlusive arterial disease. N Engl J Med. 1985;313: 709-715.
7. Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D et al. A prospective study of plasma homocysteine and risk of myocardial infarction in US physicians. JAMA. 1992;268: 877-881.
8. Graham IM, Daly LE. Refsum HM, Robinson K, Brattstrom LE, Ueland PM, et al. Plasma homocysteine as a risk factor for vascular disease: the European Concerted Action Project. JAMA. 1997;277: 1775-1781.
9. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assess-ment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. JAMA. 1995; 274(13): 1049-1057.
10. Majumder AAS, Ali MA, Saha GK. Comparison of risk factors, prevalence and complications between early onset and late onset. AMI'. BHJ. 2000;15: 77-80.
11. Siddique MA, Shrestha MP, Salman M, Haque KMHS S, Ahmed MK, Sultan M AU et al. Age-Related Differences of Risk Profile and Angiographic Findings in Patients with Coronary Heart Disease. BSMMU J. 2010;3(1): 13-17.
12. Haque AFMS, Siddiqui AR, Rahman SMM, Iqbal SA, Fatema NN, Khan Z. Acute Coronary Syndrome in the Young –Risk Factors and Angiographic Pattern, Cardiovasc J. 2010;2(2): 175-178.

North Bengal Medical College Journal
Vol 2 No 2 July 2016

Original Article

Comparison between Antibiotic Sensitivity of Community and Hospital Acquired Infections Caused by *Escherichia coli*

Taslima Yasmin,[1] Ummul Wara Khan Chowdhury,[2] Golam Mowla,[3] Shamima Akhter[4]

Revised : May 15, 2016 Accepted : May 29, 2016

Abstract

Introduction: *Escherichia coli is the most common Gram Negative Bacillus (GNB) causing various types of infection in both hospital and community. The purpose of the present study was to see the status of sensitivity of E. coli infection among hospital and community.*

Methods: *This cross sectional study was conducted in the Department of Microbiology at Mymensingh Medical College, Bangladesh from January 2011 to June 2011 for a period of 6 months. All the patients, at any age of both sexes, presented with wound infection and UTI were taken as study population. Specimens were taken aseptically. Specimens were processed and bacteria were isolated and identified according to standard procedure. Antimicrobial sensitivity test was done by disc diffusion method.*

Results: *A total number of 300 GNB were taken from various clinical specimens, among them majority was E. coli (52.0%), followed by Proteus (18.3%) and Klebsiella species (15%). All the isolates were sensitive to imipenem and nitrofurantoin followed by amikacin (92.9%).*

Conclusion: *In conclusion, E. coli is the most common bacteria causing wound infection, and UTI with a reduced sensitivity towards antibiotics both in hospital and community.*

Key words: *Escherichia coli, Gram Negative Bacilli, Antibiotic sensitivity*

North Bengal Med. Coll.J. 2016; 2 (2) : 42-49

1. *Associate Professor, Department of Microbiology, North Bengal Medical College, Sirajganj*
2. *Residential surgeon, Department of Gynae and Obstretics, Mymensingh Medical College, Mymensingh*
3. *Lecturer, Department of Community Medicine, Mymensingh Medical College, Mymensingh*
4. *Assistant Professor, Department of Pathology, TMSS Medical College, Bogra*

Correspondence *TaslimaYasmin, Email: taslimasanta@yahoo.com*

Introduction

Escheraichia coli, the most common Gram negative bacilli causing various types of infection in human. *E. coli* and related bacteria possess the ability to transfer DNA via bacterial conjugation, transduction or transformation, which allows genetic material to spread horizontally through an existing population.[1] This process led to the spread of various types of gene carried by a bacteriophage.[2] *E. coli* normally colonizes gastrointestinal tract in the bowel, it adheres to the mucus of the large intestine. It is the primary facultative anaerobe of the human gastrointestinal tract.[3] As long as these bacteria do not acquire genetic elements encoding for virulence factors, they remain benign commensals.[4] *E. coli*, when enters into unnatural sites, can cause variety of infectious diseases such as urinary tract infections, wound infections, bacteraemia, meningitis and other soft tissue infections.[5] The ability of *E. coli* to cause extra intestinal infections depends largely on several virulence factors, which help in the survival of *E. coli* under adverse conditions present in those sites, virulence factors such as haemolysin, surface hydrophobicity, serum resistance and protease.[6] In rarer cases, virulent strains are also responsible for haemolytic-uremic syndrome, peritonitis, mastitis, septicaemia and Gram-negative pneumonia.[7]

The treatment of *E. coli* infections is increasingly becoming difficult because of the multidrug resistance exhibited by the organism.[8] With the emergence and dissemination of antimicrobial resistance in bacteria which is well documented worldwide.[9] *E. coli*, an important gastrointestinal flora, known to be capable of accepting and transferring plasmids and which under stress readily transfers those plasmids to other species, is therefore considered an important reservoir of transferable antibiotic resistance.[10] Infection by *E. coli* occurs not only in hospital but also in community.[11] So it is important to find out the antibiotic sensitivity pattern of *E. coli* among hospital and community.

Materials and Methods

This cross sectional study was carried out in the Department of Microbiology at Mymensingh Medical College, Mymensingh, from January 2011 to June 2011. Patients presented with UTI or wound infection, at any age with both sexes, were taken as study population. Non-repetitive clinical isolates were collected from MMCH both the outpatients and inpatients Departments of Sugery, Medicine and Gynae over a period of 6 months. Urine, pus and wound swab were used as specimens. Laboratory work was carried out in the department of Microbiology in Mymensingh Medical College. Specimens were collected aseptically. All samples were routinely cultured on MacConkey and blood agar plates at 37^0C aerobically for 18 hours. Gram negative isolates were further characterized by standard biochemical tests.

The susceptibility to antibiotics was determined by modified Kirby Bauer method according to CLSI 2010 protocols for Gram negative panels. *Esch. coli*ATCC 25922 was used as control strains.

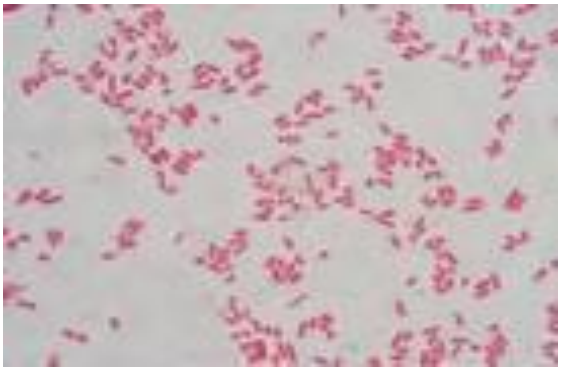

Figure 1: *Esch. coli*

Results

During 6 months period, a total of 300 Gram negative isolates from various clinical specimens were included in the study. The distribution of various specimens were shown in Table I. The specimens were as follows as urine 216 (72%), wound swab 45 (15 %) and pus 39 (13%).

Table I: Distribution of sample from various specimens

Type of specimen	Total no. (%)
Urine	216 (72%)
Wound swab	45 (15%)
Pus	39 (13%)
Total	**300 (100%)**

Out of 300 Gram negative isolates in this study majority were *Esch. coli* 156 (52%), followed by *Proteus* spp. 55 (18.3%), *Klebsiella*spp. 45 (15%), *Pseudomonas* spp. 9 (3%) and others (*Enterobacter spp.*, *Citrobacter spp*.) 35 (11.7%). (Table II).

Table II: Detection rate of different isolates in the study Population

Name of the organisms	Total No.	Percent (%)
Esch. coli	156	52.0
Proteus spp.	55	18.3
Klebsiella spp.	45	15.0
Pseudomonas spp.	9	3.0
**Others*	35	11.7
Total	**300**	**100.0**

* Others -*Enterobacter spp., Citrobacterspp*

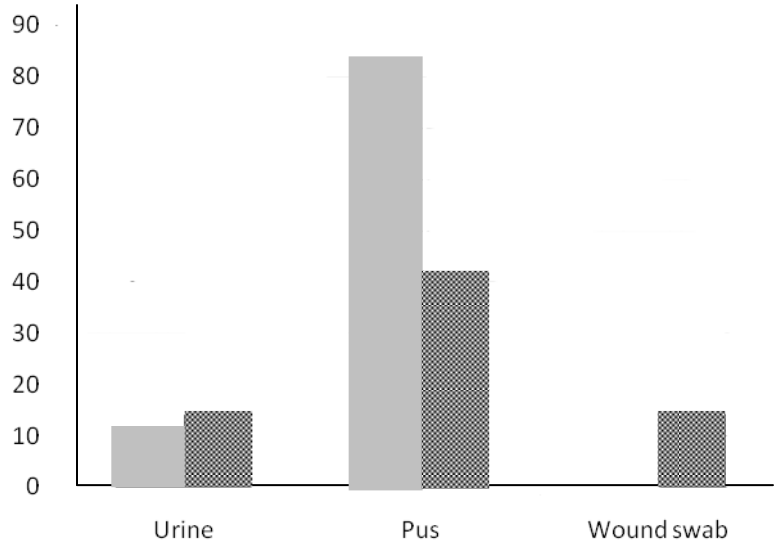

Figure 2: Distribution of *E. coli* in different specimens both from Hospital and Community

Table III: Antibiotic Resistance Pattern of *E. coli* isolated from Hospital and Community by disc diffusion method

Antimicrobials *E. coli*				
	Hospital isolates n - 67		**Community isolates n - 89**	
	Resistance	**Sensitive**	**Resistance**	**Sensitive**
Aztreonam	67 (100%)	0 (0%)	89 (100%)	0 (0%)
Piperacillin	62 (92.5%)	5 (7.5%)	85 (95.5%)	4 (4.5%)
Amoxiclav	63 (94.0%)	4 (6.0%)	83 (93.2%)	6 (6.8%)
Ampicillin	64 (95.5%)	3 (4.5%)	80 (89.8%)	9 (10.2%)
Ceftriaxone	57 (85.0%)	10 (15%)	70 (78.6%)	19 (21.4%)
Ciprofloxacin	56 (83.5%)	11 (16.5%)	64 (71.9%)	25 (28.1%)
Cefotaxime	53 (79.1%)	14 (20.9%)	63 (70.7%)	26 (29.3%)
Ceftazidime	41 (61.1%)	26 (38.9%)	63 (70.7%)	26 (29.3%)
Azithromycin	54 (80.5%)	28 (19.5%)	52 (58.4%)	37 (41.6%)
Gentamicin	39 (58.2%)	28 (41.8%)	42 (47.1%)	47 (52.9%)
Amikacin	12 (17.9%)	55 (82.1%)	11 (16.4%)	78 (83.6%)
Nitrofurantoin	2 (2.9%)	65 (97.1%)	3 (3.3%)	86 (96.7%)
Imipenem	0 (0%)	67 (100%)	0 (0%)	67 (100%)

Discussion

In the last few decades, the frequency and spectrum of antimicrobial resistant infections have increased in both the hospital and the community. Certain infections that are essentially untreatable have begun to occur as epidemics both in the developing world and in institutional developed regions.[15] Proper use of antibiotics is very important for various reasons. Development of bacterial resistance against newer antibiotics makes the main focus of research.

In the present study, a total of 300 Gram negative strains were isolated from various clinical specimens of which majority of the organisms were isolated from urine 72% followed by wound swab 15%, pus 13%. Among the isolated organisms *E. coli* was the most prevalent 52%, followed by *Proteus spp*. 18.3%, *Klebsiella spp*. 15% and *Pseudomonas spp*. 3% . In the present study isolation of *E. coli* was 55.6% and 61.2% from both urine and pus respectively, which were in agreement with the findings done by Haque et al.[13] and Parveen et al.[14] in the same institute. In India Marcus[15] and associates found the same figure.

In Bangladesh, it has been reported that 65-92% of commensal *Enterobacteriaceae* and other organisms isolated from urine is resistant to commonly used antibiotics like ampicillin, tetracycline, co-trimoxazoleetc.[16] Now-a-day's organism encoding multiple antibiotic resistance genes are becoming increasingly prevalent.[17] In this study, aztreonam, ampicillin, amoxyclave were found 95-100% resistant (Table III), this was in agreement with other studies.[18,19] Cephalosporins especially third generation have been used for Gram negative bacterial treatment.[20] In the present study ceftri-axone, ceftazidime and cefotaxime were found 85%, 61%, 79% from hospital acquired and 78.6%, 70.7%, 70.7% resistant among community acquired *E. coli* respectively. It correlates with the study done by Sasirekha et al.and Singh and Goyal (2003) in India[18,21,] where they found 84% resistance to cefotaxime and 75%, 85% resistant for ceftriaxone and ceftazidime respectively. Hospital acquired isolates were more resistant than the community acquired isolates, it may be due to lack of antibiotic policy, irrational use of 3GCs mainly ceftriaxone in the hospital Shova[22] and the emergence of antibiotic-resistant organisms in hospitals in concert with the use of high levels of antibiotics use caused the emergence of resistant organisms and they might be inherently more virulent than the organisms are sensitive (CDC 2002).

In this study observed resistance to ciprofloxacin was 78% in *E. coli*. These findings were in accordance with the study by Haque and Salam[13] from Bangladesh and it was 90.9%. Another study by Sasirekha

from India where they found 68% resistant to ciprofloxacin.[18] Aminoglycosides have good activity against clinically important gram negative bacilli[23] In the present study 82.1% isolates were susceptible to amikacin, followed by 41.8% to gentamicin, it was similar to Sasirekha et al.[21] Several studies showed that amikacin was more sensitive than gentamicin but if it is over used than it may also become resistant. In 2010 gentamicin was 59% resistant in India and 55.5% in Bangladesh.[13,18,19] These variations may be due to increased use of gentamicin, caused by selection pressure of aminoglycosides in different region.[24] Carbapenems are the drugs of choice for many infections caused by Gram positive and Gram negative bacteria.[19] In this study imipenem was 100% sensitive. These findings were similar to study done by Haque and Salam[13] but one study showed 3.1% resistant to imipenem in Bangladesh.[25] Amikacin was the second most common sensitive drug after imipenem. So, these drug resistance organisms have limited theraputic options and necessitated the increased use of carbapenems.

Conclusion

Infection by *E. coli* increasing both in hospital and community with reduced sensitivity profiles. Indiscriminate use of antibiotics should be restricted and adequate laboratory facility for culture and sensitivity should be ensured. The infection control programs should be monitored continuously in hospital.

Contribution of the Authors

First author was the principal researcher. Others were responsible for data collection, statistical analysis and computer composing.

References

1. Feng P, Weagant S, Grant M. Enumeration of Escherichia coli and the Coliform Bacteria. Bacteriological Analytical Manual, 8th ed. FDA/Center for Food Safety & Applied Nutrition. Retrieved 2007-01-25.
2. Zwadyk P. Enterobacteriaceae: Salmonella and Shigella, Intestinal pathogens. In: Joklik, W.K. Willet, H.P. Amos, B. and Wilfert CM, eds. Zinsser Microbiology, 20th ed. USA: Appleton and Lange, 1992; 556-565.
3. Todar K. Pathogenic E. coli. Online Textbook of Bacteriology. University of Wisconsin–Madison Department of Bacteriology. Retrieved 2007-11-30.
4. Evans Jr, Doyle J, Dolores G. Evans. *Escherichia coli*. Medical Microbiology, 4th edition. The University of Texas Medical Branch at Galveston. Archived from the original on 2007-11-02.
5. Rendón MA, Saladana Z, Erdem AL, Monteiro-Neto V, Vazquez A, Kaper JB et al. Commensal and pathogenic *Escherichia coli* use a common pilus adherence factor for epithelial cell colonization. PNAS 104, 2007**;** 25: 10637–42.

6. Heaton JC, Jones K. Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. J Appl Microbiol. 2008; 3: 613–26.
7. Chalmers RMH, Aird FJ. Bolton Waterborne *Escherichia* Bitzan, M and Karch, H (1992).
8. Yasmin T, Hossain M, Paul SK, Sultana S, Kabir MR, Mawla G et al. Prevalence of ESBL producing isolates among skin wound infection in a Tertiary care Hospital In Bangladesh. Mymensingh Med J. 2013; 23 (3) : 23-27.
9. Sahm DF, Thornsberry C, Mayfield DC, Jones ME, Karlowsky JA. Multidrug-resistant urinary tract isolates of *Escherichia coli:* prevalence and patient demographics in the United States in 2000. Antimicrob agent Chemother 2001;45(5): 1402-1406.
10. Chapman PA, Siddons CA, GerdanMalo AT, Harkin MA. A 1-year study of *Escherichia coli* 0157 in cattle, sheep, pigs and poultry. Epidemiol Infect. 1997;119: 245-50.
11. Akram M, Shahid M. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. Ann Clin Microbiol Antimicrob, 2007; 6 (4).
12. Aibinu IE, Peters RF, Amisu KO, Adesida SA, Ojo MO, Tol Odugbemi. Multidrug Resistance in *E. coli* 0157 strains and the Public Health Implication. J Am Sc. 2007; 3(3): 25-33.
13. Haque R, Salam MA. Detection of ESBL producing nosocomial gram negative bacteria from a tertiary care hospital in Bangladesh. Pk J Med Sci. 2010; 26(4): 887-891.
14. Parveen US, Hossain MA, Musa AK, Mahmud C, Islam MT, Haque N et al. Pattern of Aerobic Bacteria with Antimicrobial Susceptibility Causing Community Acquired Urinary Tract Infection. Mymensingh Med J. 2009;18(2):148-153.
15. Marcus N, Ashkenazi S, Yaari A, Samra Z, Livni G. Non-*Escherichia coli* versus *Escherichia coli* community-acquired urinary tract infections in children hospitalized in a tertiary center: relative frequency, risk factors, antimicrobial resistance and outcome. Pediatr Infect Dis J. 2005; 24 (7): 581-585.
16. Chowdhury MA, Yamanaka H, Miyoshi S, Aziz KM, Shinoda S. Ecology of Vibrio mimicus in aquatic environments. Appl Environ Microbiol. 1989; 55 (8): 2073-2078.
17. Perez F, Endimiani A, Hujer KM, Bonomo RA. The continuing challenge of ESBLs. Curr Opin Pharmacol. 2007;7 (5): 459-469 .
18. Sasirekha B, Manasa R, Ramya P, Sneha R. Frequency and Antimicrobial Sensitivity Pattern of Extended Spectrum β- Lactamases Producing *E. coli* and KlebsiellaPneumoniae Isolated in a Tertiary Care Hospital. Al Ameen J Med Sc. 2010; 3(4): 265-271.

19. Ullah F, Malik SA, Ahmed J. Antimicrobial susceptibility pattern and ESBL prevalence in Klebsiell-apneumoniae from urinary tract infections in the North –West of Pakistan. African J Microbiol. 2009; 3 (11): 670-680.
20. Samaha-Kfoury JN, Araj GF. Recent developments in β lactamases and extended spectrum β lactamases. Bairut Med J.2003; 327 (22): 1209-1213.
21. Singh NP, Goyal R. Changing trends in bacteriology of burns in the burns unit, Delhi, India. Burns. 2003; 29 (2): 129-132.
22. Shobha KL, Gowrish RS, Sugandhi R, Sreeja CK. Prevalence of Extended Spectrum β Lactamases in Urinary Isolates of *Escherichia coli*, Klebsiella and Citrobacter Species and their Antimicrobial Susceptibility Pattern in tertiary care hospital. Indian J Practic Doctor. 2007; 3(6): 1 -2.
23. Gonzalez LS, Spencer JP. Aminogly-cosides: a practical review. Am Fam Physician. 1998; 58 (8): 1811-1820.
24. Miller GH, Sabatelli FJ. The most frequent aminoglycoside resistance mechanisms--changes with time and geographic area: a reflection of aminoglycoside usage patterns? Aminoglycoside Resistance Study Groups. Clin Infect Dis.1997; 24 (1): 46-62.
25. Rashid A, Chowdhury A, Rahman SH, Begum SA, Muazzam N. Infections by Pseudomonas aeruginosa and Antibiotic Resistance Pattern of the Isolates from Dhaka Medical College Hospital. Bangladesh J Med Microbiol. 2007; 1(2): 48-51.

North Bengal Medical College Journal
Vol 2 No 2 July 2016

Review Article

Equations for Glomerular Filtration Rate Estimation

Md. Shariful Haque,[1] Shaheen Akter,[2] Harun Ur Rashid,[3] Muhammad Rafiqul Alam[4]

Revised : February 20, 2016 Accepted : March 28, 2016

Abstract

Different authorities and guidelines recommend creatinine based Glomerular Filtration Rate (GFR) estimation (eGFR) besides measurement of creatinine. In the last few years it has become routine to estimate and classify kidney function using equations based on serum creatinine. Creatinine alone is not enough detecting early stage of renal failure in Acute Kidney Injury (AKI) and Chronic Kidney Disease (CKD). A decrease in GFR precedes the onset of renal failure. Creatinine based GFR estimation equations are useful in staging of CKD, monitoring disease progression and dose adjustment in impaired renal function. Among various GFR estimation equations Cockcroft- Gault and Modification of Diet in Renal Disease (MDRD) equations are commonly used. The 2009 Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation is new and has overcome some limitations of other equations. In this article we discuss different commonly used equations.

Key words: *eGFR, CKD, MDRD*

North Bengal Med. Coll.J. 2016; 2 (2) : 50-57

1. *Assistant Professor, Nephrology, Shaheed M. Monsur Ali Medical College, Sirajganj*
2. *Associate Professor, Pathology, North Bengal Medical College, Sirajganj*
3. *Professor of Nephrology, Kidney Foundation, Mirpur-2, Dhaka-1216*
4. *Professor of Nephrology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka*

Correspondence *Md. Shariful Haque, Email: sharifuldr@gmail.com*

Introduction

The Glomerular Filtration Rate (GFR) is traditionally considered the best overall index of kidney function**.** In 2002, the National Kidney Foundation (NKF) Kidney Disease Outcome Quality Initiative (K/DOQI) clinical practice guidelines on chronic kidney disease recommends estimating the level of GFR by employing prediction equations that incorporate serum creatinine measurements.[1] This method of determining GFR was judged preferable to using serum creatinine alone or measuring creatinine clearance. Gold standard tests for measurement of renal function include the inulin clearance assay and the 125I-iodothalamate clearance assay. Unfortunately, performing these tests is difficult, and they are not available in most medical laboratories. Instead, the focus has traditionally been on serum creatinine level and on the 24 hours creatinine clearance. The National Kidney Disease Education Program (NKDEP) of the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) in United States. National Kidney Foundation (NKF) of United States and American Society of Nephrology (ASN) recommend estimating GFR (eGFR) from serum creatinine. Estimated GFR is important in assessing the excretory function of the kidneys. For example, grading of chronic renal insufficiency and dosage of drugs that are primarily excreted via urine are based on GFR (or creatinine clearance).

Methods of creatinine measurement influence GFR estimation. Several mathematical formulae have been developed to estimate GFR (eGFR) from the creatinine concentration and parameters such as the patient's age and sex. Jelliffe (1971), Mawer (1972), Jelliffe (1973), Cockcroft -Gault (1976), Hull (1981), Bjornsson (1983), Gates (1985), Levey (1999), Levey (2000)and many other equations are available. Most widely used are the Cockcroft Gault (CG) and the Modification of Diet in Renal Disease (MDRD) formulae.[7] Subsequent studies have shown that CG tends to over-estimate renal function, especially, at lower levels[4] whilst MDRD under-estimates GFR particularly at near normal levels of function. Several studies[10] have demonstrated the superiority of MDRD for screening in a variety of different populations and it is now advocated as the method of choice in the UK (Department of Health, 2005).

A new equation, the CKD-EPI (CKD Epidemiology collaboration) equation by Andrew S Levey in 2009 is better than others in terms of accuracy, less bias and applicability to all races. So the goal is to evaluate the number of commonly used equations for GFR.

Discussion

Creatinine is not a very sensitive marker at early stage of renal failure. A decrease in GFR precedes the onset of renal failure. Estimated GFR (eGFR) by different equations obviates need of GFR measurement in many cases. There are no fewer than 46 different prediction equations currently available, although the two most commonly used are the Cockcroft-Gault and the "Modification of Diet in Renal Disease" (MDRD) formulae.[2] CKD Epidemiology Collaboration (CKD EPI) formula is the latest and claimed to be better than all others, and is being used in every countries including Bangladesh.

Cockcroft-Gaultformula[3]

The first more widely spread formula used to estimate renal function is the Cockcroft-Gault formula.

GFR (ml/min) = $\frac{(140\text{-age in years}) \times \text{weight (in kg)} \times (0.85 \text{ if female})}{72 \times \text{S.Cr (mg/dl)}}$

The Cockcroft and Gault formula was developed in 1973 (published in 1976), using data from 249 men aged 18-92 with creatinine clearance (C_{Cr}) from approximately 30 to 130 mL/m^2. The Cockcroft-Gault formula estimates the creatinine clearance, which is not corrected by body surface area, and thus the absolute value of the filtration rate. Due to the increased creatinine secretion, the creatinine clearance usually overestimates GFR when the GFR is low. There are several limitations to the C-G equation. The reference GFR was the 24-hours-urine creatinine clearance (C_{Cr}), so the predicted value of C-G equation is actually surrogate of creatinineclearance rate.In most of the comparative analysis performed; it is shown that the classical CG equation overestimated glomerular filtration.[4, 5] The sample size was small and all of them were male, 15% reduction was proposed for women.

MDRD (Modification of Diet in Renal Disease) study equation[6, 7]

The MDRD (Modification of Diet in Renal Disease) Study equation was developed in 1999 using data from 1,628 patients with CKD with GFR from approximately 5 to 90 ml/min/1.73 m^2. It estimates GFR adjusted for body surface area and is more accurate than measured creatinine clearance from 24-hours urine collections or estimated by the Cockcroft and Gault formula. The formula was developed from the MDRD study, which consisted of non-transplanted CKD-patients with non-diabetic renal disease.

The abbreviated, or four-variable equation includes age, sex, creatinine, and race (black or not black). Adding more variables (albumin, urea) adds little to accuracy.

4 variable MDRD equation:

eGFR =186 x $(SCr)^{-1.154}$ x $(age)^{-0.203}$ x (0.742 if female) x (1.210 if black)

6 variable MDRD equation:

$GFR=170x(SCr)^{-0.999}x\ (age)^{-0.176}\ x[0.762$ if female]x [1.180 if black] x $BUN^{-0.170}$ x $Albumin^{+0.318}$

The MDRD equation was re-expressed in 2006. The new equation is adjusted to a standardized creatinine calibration which gives approximately 5% lower values of the S-Cr.[8, 9]

Re-expressed MDRD equation

GFR = 175 x (Standardized S-Cr)$^{-1.154}$ x (age)$^{-0.203}$ x (0.742 if female) x (1.210 if African American)

For individuals of African or Caribbean descent the eGFR value obtained should be multiplied by a factor of 1.2 to reflect a greater inherent muscle mass. All other races are to use the basic formula provided. The MDRD does not require the input of height or weight variables and avoids inaccuracies introduced through these measures. It also has been standardized to a 1.73 m^2 body surface area (BSA) which is the normal average for young adults. Adjusting the formula for BSA allows comparisons of an individual's result with age/sex/race appropriate norms. In doing so it also provides a convenient means to compare an individual's renal function to defined stages of CKD.

The MDRD formula is not considered applicable to individuals age <18 years, those with rapidly changing kidney function such as individuals experiencing acute renal failure, pregnant females, oedematous states such as Congestive Heart Failure(CHF), individuals with muscle wasting diseases-rhabdomyolysis, recent trauma, amputees, paraplegics, quadriplegics, malnourished individuals and those at both extremes of body size (BMI). In the above circumstances, a 24 hours creatinine clearance is acknowledged to provide a better estimate of the GFR. MDRD study equation has been derived based on GFR measured using an accepted method (urinary clearance of 125I-iothalamate or traceable to an isotope dilution mass spectrometry (IDMS) reference method, hence, it estimates GFR rather than creatinine clearance.

Several studies have shown that in "low-risk" populations, such as living kidney donors or individuals with early diabetes, the MDRD equation systematically underestimated GFR, particularly in patients with high-normal serum creatinine levels.[10,11] One caveat is that the MDRD formula tends to provide falsely low estimates in young healthy individuals (especially well muscled males).[12] In fact, some authorities suggest routine reporting of specific eGFR values >60 ml/min/1.73 m^2 is not recommended.[13] Again, this issue arises as the MDRD has not been validated for screening in healthy normal individuals. Its use should be restricted to screening those at

risk of CKD and those with known CKD to gauge severity and ascertain prognosis.[14] Rule AD found the MDRD equation underestimated GFR by 6.2% in patients with chronic kidney disease and by 29% in healthy persons.[11]

Many studies have compared the performance of the two equations to measured GFR. In some of these studies, the MDRD Study equation was more accurate than the Cockcroft and Gault equation. Other studies demonstrated similar performance. The Cockcroft and Gault equation appears to be less accurate than the MDRD Study equation, specifically in older and obese people.[15]

The Mayo Clinic's Quadratic Equation

The Mayo clinic quadratic equation is a new equation developed by Rule AD and Larson TS et.al. at the Mayo Clinic, a tertiary-care medical centre based on the results of iothalamate clearance in both 320 patients with chronic kidney diseases and 580 healthy subjects evaluated for kidney donation .

The Mayo quadratic equation was further shown to have similar diagnostic performance to the MDRD equation in diabetic patients; in contrast to MDRD equation, the Mayo quadratic equation does not underestimate normal GFR in diabetic subjects.[16]

CKD-EPI Formula

The CKD Epidemiology Collaboration (CKD-EPI) equation was published in 2009 and intended to be more generalizable across various clinical settings than the MDRD equation. It has developed from a large database of participants in research studies and patients from clinical populations with diverse characteristics including those with or without kidney disease, diabetes and history of organ transplantation to overcome limitations of the MDRD study equation. CKD-EPI produces higher eGFR values in the high eGFR range (>60 ml/min/1.73 m2), and lower eGFR values in the lowest range.[17] Weight, diabetes, and transplant were considered as potential variables, but the final equation uses the same variables as the MDRD equation.[18] By CKD-EPI equation median estimated GFR was 9.5 ml/min/ 1.73 m^2 higher, which decreases the prevalence estimate for chronic kidney disease by 1.6% (11.5% vs. 13.1% using the MDRD Study equation) in US population.

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula developed is represented as the equation below, in which the values of the constants of *a*, *b*, and *c* vary on the basis of race, sex, and serum creatinine.

$$\mathbf{GFR} = a \times (\text{serum creatinine}/b)^{c} \times (0.993)^{\text{age}}$$

The variable *a* takes on the following values on the basis of race and sex:

- Black
  - Women = 166
  - Men = 163
- White/other
  - Women = 144
  - Men = 141

The variable *b* takes on the following values on the basis of sex:

- Women = 0.7
- Men = 0.9

The variable *c* takes on the following values on the basis of sex and creatinine measurement:

- Women
  - Serum creatinine ≤ 0.7 mg/dL = -0.329
  - Serum creatinine> 0.7 mg/dL = -1.209
- Men
  - Serum creatinine ≤ 0.9 mg/dL = -0.411
  - Serum creatinine> 0.9 mg/dL = -1.209

Authors concluded: The CKD-EPI creatinine equation is more accurate than the MDRD. Study equation and could replace it for routine clinical use. The 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines recommend that clinical laboratories report eGFR in all adults using CKD-EPI creatinine equations, or using other equations if shown to be superior to CKD-EPI equation in that population.[19]

Schwartz's Formula

Schwartz and colleagues[20]originally published a formula to estimate glomerular filtration rate (GFR) in children in 1976, the same year that Cockcroft and Gault published what became the canonical estimation formula for creatinine clearance in adults.

Original Schwartz Formula

GFR (mL/min/1.73 m^2) = k× (Height) / Serum Creatinine

k = Constant

o k = 0.33 in Preemie Infants

o k = 0.45 in Term infants to 1 year old

o k = 0.55 in Children and adolescent girls

o k = 0.65 in Adolescent males (Not females because of the presumed increase in male muscle mass. The constant remains 0 .55 for females.) Height in cm,Serum Creatinine in mg/dl.

A recent report by Schwartz and coworkers has described improved equations for estimating GFR in children [21]. The equations were developed with data collected from 349 children 1–16 years of age with GFRs of approximately20–90 ml/min/1.73 m^2 who were enrolled in the Chronic Kidney Disease in Children (CKiD) study. Like the original equation, the revised equationis based on height and creatinine measurements, but the new equation uses creatinine as measured by an enzymatic method with calibration traceable to an isotope dilution mass

spectrometry (IDMS) reference measurement procedure. This updated Schwartz equation has not been validated for use with methods based on the Jaffe (alkaline picrate) reaction.

Revised Schwartz's Equation:

GFR (mL/min/1.73 m^2) = (0.41 × Height in cm) / Creatinine in mg/dL

Conclusion

Pursuit for correcting GFR is continuing. GFR estimating equations using serum Cystatin C, ßTP (ß Trace Protein), $ß_2M$ ($ß_2$Microglobulin) has been developed. Until now CKD EPI equation is best recommended for estimating GFR based on creatinine measurement. Application based soft wares for estimation of GFR are available for smart devices. Clinical laboratories and doctors should routinely use creatinine based eGFR besides other tests of kidney function.

References

1. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification and stratification. Am J Kidney Dis. 2002;39(1): S1-S266.
2. Diamandopoulos A, Goudas P, Arvanitis A. Comparison of estimated creatinine clearance among five formulae (Cockroft–Gault, Jelliffe, Sanaka, simplified 4 variable MDRD and DAF) and the 24 hours-urine-collection creatinine clearance. HIPPOKRATIA. 2010,14(2): 98-104.
3. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1): 31-41.
4. Lin J, Knight EL, Hogan ML, Singh AK. A comparison of prediction equations for estimating glomerular filtration rate in adults without kidney disease. J Am Soc Nephrol. 2003;14: 2573-2580.
5. Poggio ED, Wang X, Weinstein DM, Issa N, Dennis VW, Braun WE et. al Assessing glomerular filtration rate by estimation equations in kidney transplant recipients. Am J Transplant. 2006: 100-108.
6. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D for the Modification of Diet in Renal Disease Study Group. A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Ann Intern Med. 1996;130(6): 461-470.
7. Levey AS, Greene T, Kusek JW, Beck GJ, MDRD Study Group. A simplified equation to predict glomerular filtration rate from serum creatinine. J Am Soc Nephrol.2000;11: A 08-28.
8. Miller W, Myers G, Ashwood ER, Killeen AA, Wang E, Theinpont LM, et al. Creatinine measurement: State of the art in accuracy and interlaboratory harmonization. Arch Pathol Lab Med. 2005;129: 297-304

9. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW et al. Expressing the Modification of Diet in Renal Disease. Study Equation for estimating Glomerular Filtration Rate with Stanfardized Serum Creatinine Values. Clinical Chemistry 2007; 53 (4): 766-772
10. Ibrahim H, Mondress M, Tello A, Fan Y, Koopmeiners J, Thomas W: An alternative formula to the Cockcroft-Gault and the Modification of Diet in Renal Disease formulas in predicting GFR in individuals with type 1 diabetes. J Am Soc Nephrol. 2005;16: 1051–1060.
11. Rule AD, Larson TS, Bergstralh EJ, Slezak JM, Jacobsen SJ, Cosio FG: Using serum creatinine to estimate glomerular filtration rate: Accuracy in good health and in chronic kidney disease. Ann Intern Med. 2004;141: 929–937.
12. Poggio ED, Wang X, Greene T, Van Lente F, Hall PM. Performance of the Modification of Diet in Renal Disease and Cockcroft-Gault equations in the estimation of GFR in health and in chronic kidney disease. J Am Soc Nephrol. 2005; 16: 459–466.
13. Richardson R. Estimated Glomerular Filtration Rate–Use and Abuse. CLIMOA. 2007 proceedings 2008 spring; volume 4.
14. Richard Rougeau. eGFR: Estimated Glomerular Filtration Rate. Generali USA. 2008 spring; Volume 4.
15. Coresh J, Stevens LA. Kidney function estimating equations : where do we stand ? Curr Opin Nephrol Hypertens. 2006; 15 (3): 276-284
16. Rigalleau V, Lasseur C, Raffaitin C, Perlemoine C, Barthe N, Chauveau P et al. The Mayo clinic quadratic equation improves the prediction of glomerular filtration rate in diabetic subjects. Nephrol Dial Transplant. 2007; 22: 813-818.
17. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro AF, Feldman HI et al. Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI): A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009; 150: 604-612.
18. Stevens LA, Schmid CH, Zhang YL, Coresh J, Rule AD, Kusek J et al: Development and validation of GFR estimating equations using diabetes, transplant and weight. Nephrol Dial Transplant. 2009;25: 449–457.
19. KDIGO 2012 clinical practice guidelines for evaluation and management of chronic kidney disease. Kidney Int Suppl. 2013; 3 (1) : 5-14
20. Schwartz GJ, Haycock GB, Edelmann CM, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics. 1976;58: 259–263
21. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA et. al New equations to estimate GFR in children with CKD. J Am Soc Nephrol. 2009, 20: 629-637

North Bengal Medical College Journal
Vol 2 No 2 July 2016

Case Report

Situs Inversus Totalis

SM Nazim Uddin,[1] Md. Nayeem Ullah,[2] Md. Liaquat Ali,[3] Chowdhury M Khoda[4]

Revised : May 06, 2016 Accepted : May 29, 2016

Abstract

A 2 months old girl weighing 3.2kg, 1st issue of a non-consanguineous parents immunized as per EPI schedule was born at term at home by NVD presented to the emergency department of NBMCH with severe respiratory distress and fever for 5 days. Her mother also complained of unable to feed for 1 day. For routine examination, she was referred to the Medical Imaging Center for X-ray chest & abdomen, USG of whole abdomen, echocardiography and some radiographic studies like CT scan etc. But unfortunately, we could not done CT scan due to unavailability. However, by the USG and X-ray we observed a situs inversus totalis. We found right sided heart, stomach and spleen as well as left sided liver with gall bladder. Her parents were unaware about her unusual anatomy.

Key words: *Situs inversus totalis, Dextrocardia, Congenital abnormality, Transposition of viscerae.*

North Bengal Med. Coll.J. 2016; 2 (2) : 58-62

1. *Registrar,Department of Paediatrics, North Bengal Medical College, Sirajgan.*
2. *Assistant Professor, Radiology & Imaging Department, North Bengal Medical College, Sirajganj*
3. *Assistant Professor, Department of Paediatrics, North Bengal Medical College, Sirajganj*
4. *Associate Professor, Department of Paediatrics, North Bengal Medical College, Sirajganj*

Correspondence *S M Nazim Uddin, Email:dr.nujewel@gmail.com*

Introduction

Situs inversus totalis is a congenital positional anomaly characterized by transposition of abdominal viscera associated with a right sided heart (Dextrocardia). Situs inversus totalis is a condition in which the organs of the chest and abdomen are arranged in perfect mirror image of normal.[3] Both male & female ratio is 3:2.[13]

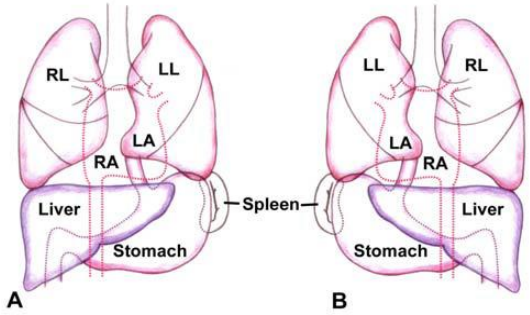

Figure 1: Schematic Diagram of Mirror Image of Situs Inversus
(Adapted from Wilhelm A, 2011)[3]

It was Mathew Baillie who first described situs inversus totalis in early twentieth century. A transposed thoracic and abdominal organ is a mirror image of the normal anatomy confirmed by radio imaging such as x-ray film and USG of abdomen. Generally individuals with situs inversus totalis are asymptomatic and have a normal life expectancy. Many people with situs inversus totalis are unaware of their unusual anatomy until they seek medical attention for an unrelated condition. The reversal of the organs may lead to some confusion as many signs and symptoms are found on the reverse side.

The prevalence of situs inversus varies among different populations which is less than 1 in 10,000 population. Situs inversus is a rare congenital anomaly characterized by transposition of the abdominal organs, viscera& vasculature. When associated with dextrocardia, it is known as situsinversus-totalis.[1] This condition is generally an autosomal recessive genetic condition. It may or may not be associated with dextrocardia.[1,2] Generally genetic anomaly is discovered incidentally when radiographic assessment of patient is performed. The heart is located on the right side of the thorax, the stomach and spleen are also located on the right side of the abdomen and the liver and gall bladder on left.

The situs inversus with dextrocardia or situs inversus totalis has been occurred once in about 6000-8000 live births. Situs inversus with levocardiaorsitus inversus incompletes is an another rare condition (1 in 22,000 of general population) in which the heart is found on the normal left side of the thorax[6]. Situs inversus with levocardia or dextrocardia without situs inversus present with much higher rates in congenital defects than situs inversus with dextrocardia. We report this case of situs inversus discovered in the medical imaging center following image studies compared with normal anatomy in relation to severe pneumonia.

Discussion

Situs inversus is generally an autosomal recessive genetic condition, sometimes[12] it can be X-linked and also found in identical twins[10,16] and there is no difference between races.

Recent studies suggest that left-right asymmetry defects is to be due to genetic abnormalities in lefty genes, nodal genes, and ZIC 3, ACVR2B and Pitxz genes. Mutation of genes present on chromosome 12.[12,15] In the absence of congenital heart defects, individuals with situs inversus are phenotypically un impaired and can live normal healthy life without complications (Figure 2). About 25% of individuals with situs inversus have an underling condition known as primary ciliary dyskinesia (PCD). Situs inversus with PCD together known as "Kantagener Syndrom".[14]

.

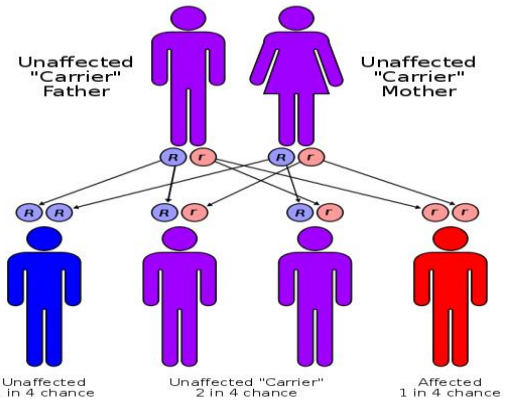

Figure 2. Situs Inversus has an Autosomal Recessive pattern of inheritance
(Adapted from Kosaki et al, 1999)[12]

Situs inversus occurs more commonly with dextrocardia.[3] A 3%-5% incidence of congenital heart disease is observed in situs inversus with dextrocardia, usually with transposition of the great vessels, 80% of these patients have a right-side aortic arch.

Situs inversus with levocardia rare[5] and it is almost always associated with congenital heart disease.[6,7,8,9] Many people with situs inversus totalis are unaware of their unusual anatomy until they seek medical attention for unrelated condition. The reversal of the organs may then lead to some confusion, as many signs and symptoms would be on the wrong side.

For example, if an individual with situs inversus develops appendicitis, they will present to the physician with lower abdominal pain in the left side instead of right where the appendix generally lies. Physician may confuse in such situation.

Abdominal and chest X-ray ultrasonography and CT scan also facilitate reliable and early diagnosis if patient is unaware of his unusual anatomy. In patient with situs inversus totalis, who present with an acute abdominal pain having appendicitis that involving left ileac region, the diagnosis become more complex and difficult. Until 2008 fewer than 10 cases of appendicitis associated with situs inversus were reported in the literature.[11] Thus, in the event of a medical problem, the knowledge of situs inversus can expedite diagnosis.

The occurrence of situs inversus is very rare. A few cases have been reported in the literature which causes diagnostic problem that can be solved by the medical image particularly X-ray chest and abdomen, USG

of the abdomen, echocardiography and CT scan. These procedures allows for early management of the disease and guidance for the best approaches.

The case

A 2 month old girl weighing 3.2 kg, 1st issue of non-consanguineous parents immunized as per EPI schedule was born at term at home by NVD presented to the emergency department of NBMCH with severe respiratory distress and fever for 5 days. Her mother also complains of unable to feed the baby for last 1 day. With above these complaints she was admitted in the department of Pediatrics.

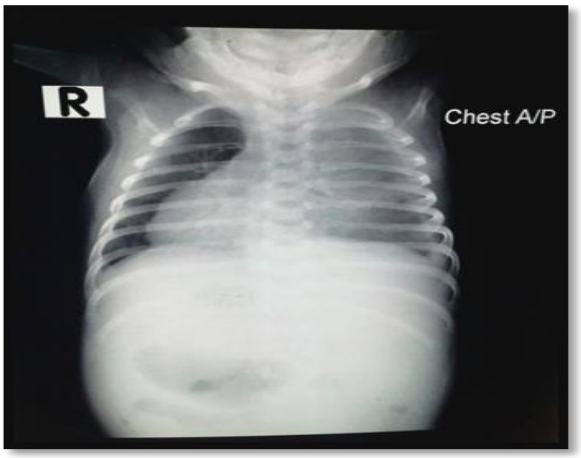

Figure-3: X-ray of Chest and Abdomen

On clinical examination, we found the patient is dysphonic, HR-120/min,Temp-102^0F, RR-66 breath/min, chest in drawing and fast breathing present, mildly anemic, non-icteric, mild-cyanotic, Heart- s_1+s_2 audible at right side of chest, lungs-rhonchi present, other clinical signs reveled normal.

We have done only chest X-ray and USG. But when we proceed to listen the heart sound it was not found in left side .On curiosity, further checked in the right side it was clearly audible with shifted apex beat. She was provisionally diagnosed as a severe pneumonia with consolidation. As the size of the chest of baby was small, we were confused about the position of the heart of the baby. Then we referred him to medical imaging center for chest X-ray, USG of whole abdomen, echocardiography and CT scan etc. But patient party could not be done CT scan and echocardiography due to unavailability and financial incapability.

We observed dextrocardia with situs inversus which is known as situs inversus totalis. Here we found right sided heart, stomach and spleen as well as left sided liver with gall bladder. All the thoracic and abdominal organ and viscera were completely inversed.

Conclusion

Patient with situs inversus who present to the junior doctor at emergency department may have diagnostic problems in physical examination because of their unusual anatomy. It is important to inform senior and expert medical personnel to diagnose a case like situs inversus totalis in order to decrease errors and prevent complications that arise during patient assessment and care, particularly in case of appendicitis and other abdominal organ anomalies. Both practical experience and theoretical knowledge play a vital role to diagnose such abnormality and always needs interdepartmental co-operation to manage the complications effectively and efficiently.

References

1. Nelson MJ, Pesola GR. Left lower quadrant Pain of Unusual Cause, J Emerg Med. 2001;20: 241-245.
2. Kassi A, Couassij, Souagak, Koffi E, Kassanyous. Appendiciteaiguesur situs inversus: Uniform etopographique a ne pas meconnaitre a propos d"uncas. Medicined"Afrique Noire. 2004,51: 249-331.
3. Wilhelm A, Situs Inversus Imaging 2011 [cited 2011 9/23]; Available from : http//emedicine.mediscape.com/article/ 413679-overview.
4. Maldjian PD, Saric M. Approach to dextrocardia in adult: Review. Ajr Am J Roentgenol. 2007;188: s39-49.
5. Gindes L, Heges J, Barkai G, Jacabson JM. Achiron R. Isolated levocardia : Prenatal diagnosis, clinical importance , and literature review. J Ultrasound Med. 2007;26: 361-365
6. Douglas Yl, jongbloed MR, Den Hartog Wc, Bartelings MM, Bogers AJ, Ebels T, et al. Pulmonary vein and atrial wall pathology in human total anomalous pulmonary venous connection . Int J Cardiol. 2009;134: 302-312
7. Fung TY, Chan DL, Leung TN, Leung TY, Lau TK. Dextrocardia in pregnancy: 20 years'experience. J Repord Med. 2006; 51: 573-577.
8. Palumbo E. Neonatal diagnosis of primary ciliary dyskinesis. Recent advances. Recent Iprog Med. 2008;99: 207-209
9. Van mierop LH, Eisen S ,Schiebler Gl. The radiographic appearance of the tracheobronchial tree as an indicator of visceral situs. Am J Cardiol. 1970;26: 432-435.
10. Gedda L, Sciacca A, Brenci G, Villatico S, Bonanni G, Gueli N, et al. Situs viscerum specularis in monozygotic twin. Acta Genet Med Gemellol (roma). 1984;33: 81-85
11. Huang SM, Yao CC, Tsai TP, Hsu GW. Acute appendicitis in situs inversus totalis. J Am Coll Surg. 2008;207: 954.
12. Kosaki R, Gebbia M, Kosaki R, Lewin M, Bowers P, Towbin JA et al. Left-right axis malformations associated with mutations in ACVR2B, the gene for human activin receptor type IIB. Am J Med Genet. 1999;82: 70-76.
13. Ryan AK, Blumberg B, Rodriguez-Esteban C, Yonei-Tamura S, Tamura K, Tsukey-T et al. Pitx2 determines left-right asymmetry of internal organs in vertebrates. Nature. 1998;394: 545-551.
14. Piedra ME ,Icardo JM, Albajar M, Rodriguez-Rey JC, Ros MA. Pitx2 participates in the late phase of the pathway controlling left-right asymmetry. Cell. 1998;94: 319-324;5043-5048.
15. Heymer J, Kuehm M, Ruther U. The expression pattern of nodal and lefty in the mouse mutant Ft suggests a function in the establishment of handedness. Mech Dev. 1997;66: 5-11 .
16. Yokoyama T, Copeland, Jenkis NA, Montgomery CA, Elder FF, Overbeek PA. "Reversal of left- right asymmetry: a situs inversus mutation". Science 1993, 260 (5108): 679-682.

North Bengal Medical College Journal
Vol 2 No 2 July 2016

Case Report

Dent's Disease: A Rare X-linked Kidney Disease

Salma Jahan,[1] Khaza Habib Salim,[2] Ferdous Jahan,[3] Md.Saiful Islam[4]

Revised : January 09, 2016 Accepted : January 23, 2016

Abstract

Dent's disease is a rare X-linked recessive proximal tubulopathy. It is typically characterized by low-molecular-weight (LMW) proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, hypophosphatemia, rickets and slowly progressive renal failure. The laboratory and clinical features may occur in various combinations. The early diagnosis of Dent's disease is often problematic because affected children may have mild clinical and biochemical signs, detecting LMW proteinurea is not available in many laboratories, and genetic results are not clear in all cases. We report on a four months old boy with nephrocalcinosis having one sister and a brother died earlier of same disease process.

Key words: *Dent's disease, Nephrocalcinosis, Proximal tubulopathy*

North Bengal Med. Coll.J. 2016; 2 (2) : 63-69

1. *Senior Consultant of Paediatrics, Centre for Woman and Child Health, Ashulia, Dhaka*
2. *Assistant Professor of Paediatric Surgery, Shaheed TajuddinAhmed Medical College, Gazipur*
3. *Medical Officer, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka*
4. *Associate Professor of Urology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka*

Correspondence *Salma Jahan, Email: sjshally@yahoo.com*

Introduction

Genetic basis of nephrocalcinosis and renal failure is suspected when number of cases cluster occur in a family. The intention of this case report arises when three sibling of a same family died with same kind of kidney disease. With all limitations of investigations and treatment facilities in our centre, we provisionally diagnosed the case as Dent's Disease for further discussion and attention.

Dent's disease is now the generally accepted name for a group of X-linked renal tubular disorders, including X-linked recessive nephrolithiasis with renal failure, X-linked recessive hypophosphatemic rickets, and idiopathic low-molecular weight (LMW) proteinurea.[1–3] Dent's disease is caused mainly by mutations in the CLCN5 gene (Dent's disease 1) located on chromosome Xp11.22, which encodes for the 746amino-acid ClC-5 chloride channel implicated in the tubular endocytoticabsorption of albumin and LMW proteins. ClC-5 was first thought to provide a shunt conductance in early endosomes, enabling efficient intraluminal acidification by V-type H + ATPase.[4–6]It has recently beendemonstrated, however, that ClC-5 functions as a Cl^-/H^+antiporter when activated by positive voltages.[7,8] No CLCN5 gene mutations are detected in approximately 40% of patients with the classic symptoms of Dent's disease, suggesting a locus heterogeneity. The OCRL gene located on chromosome Xq26.1, whose mutations cause Lowe syndrome, has recently been found altered in 20% Dent's patients,[9] but about 20% of patients carry neither CLCN5 nor OCRL mutations[10–13]. Dent's disease tends to become manifest in childhood or early adult life. It is characterized by LMW proteinuria, hypercalciuria, medullary nephrocalcinosis, nephrolithiasis, other tubular dysfunctions, and renal failure in various combinations. Different groups of researchershave recently reported on case series of patients with atypical or rare Dent,s disease 1 phenotypic signs, such as episodeic night blindness,[14] Bartter-like syndrome,[15,16] growth hormone deficiency,[17] and proteinuria with histological evidence of focal segmental glomerulosclerosis.[18,19] Dent's disease patients carrying OCRL gene mutations (Dent's disease 2) have none of the classic symptoms accompanying renal tubulopathy in Lowe syndrome, that is mental retardation, bone disease, growth retardation, congenital cataracts, delayed motor milestones. This milder phenotype is not attributable to less severe changes in protein expression or enzyme activity, as both are significantly reduced or absent.[10–13] The renal symptoms of Lowe syndrome are very similar to those of Dent's disease, though the characteristics of the patients' tubular dysfunction may differ. Recently however, two different OCRL mutations each causing both Dent's disease 2 and Lowe syndrome even in the same family have been described.[20] In short, Dent's disease seems to be characterized by a genetic and phenotypic heterogeneity. A few

studies were reported regarding Dent's disease.

Here, we have discussed an atypical case of nephrocalcinosisinvolving a syndromic variant of Dent's disease, without documented CLCN5 and OCRL mutations.

The Case

A male baby of 4months with fever, vomiting, with frequency of urination and progressive loss of weight was referred to Centre for Women and Child Health Hospital, Ashulia, Dhaka, for evaluation and better management. The full term healthy male boy with 2.9 kg weight delivered with Lower uterine Caesarean section (LUCS). From the12 days of age, he had intermittent fevers and recurrent urinary tract infection with failures to gain weight. On admission, his body weight was measured 3.2 kg, and height 52 cm.His BP was recorded as 70/50 mm Hg. He was malnourished, anaemic, mild peripheral edema with normal cardio-respiratory examination; and no rashes or arthritis. The abdomen was soft and non tender. The liver was not enlarged, and the spleen was palpable two finger breadths below the costal margin. He had low grade fever, severe dehydration with respiratory distress and was managed accordingly with intravenous fluid and antibiotics. But the patient was died on 3rd days of admission. His urine analysis shows proteinuria, plenty of pus cells with no growth of microorganism on urine culture. He had 2.1 mg/dl of serum creatinine, 8.6 mg/dl of serum calcium, 5.5 mmol/l of serum potassium, and normal serum inorganic phosphate. His complete blood count showed 8.8 gm/dl Hb, raised ESR and leukocytosis. Renal ultrasonography showed, both the kidneys were enlarged in size (right kidney was 4.5 cm, left kidney was 5.0 cm), but normal in shape and position. Cortico-medullary differentiation was well, pelvicalyceal system were not dilated. Multiple echogenic structures seen at both kidneys which were arranged radially with feature of nephrocalcinosis. (Figure 1)

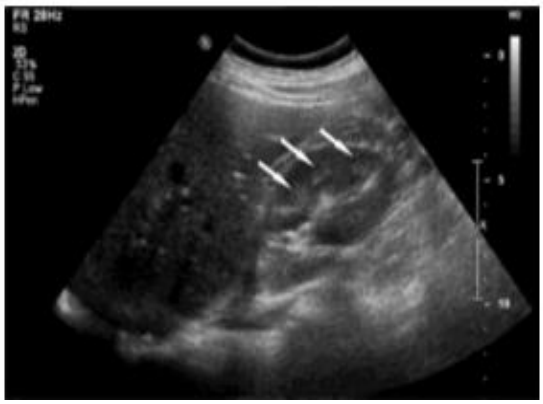

Figure 1: Ultrasound of the Right Kidney

Family history revealed that parents of the patient were maternal cousin. They were two brothers and one sister. His older brother died at the age of 5 months due to CKD with nephrocalcinosis. His elder sister died at the age of 1 year with progressive microalbuminuria, hyperkalemia and medu-llary nephrocalcinosis.

Renal biopsy of brother or sister shows mild focal global glomerulosclerosis, tubular atrophy, and interstitial fibrosis with multiple medullary tubular calcifications, consistent with Dent's disease. Gene analysis was not done due to short survival and non-availability of test facility.

Discussion

Dent's disease is a rare recessive X-linked renal tubular disorder manifesting byFanconi's syndrome or proximal tubular dysfunction of different grades, nephro-lithiasis, nephrocalcinosis, rickets and slowly progressive renalfailure. Its precise prevalence is unknown and approximately 250 affected families were reported worldwide. In the past, several phenotypic variants of Dent's disease were independently described and named as separate disorders, including X-linked recessive nephrolithiasis with renal failure, X-linked recessive hypophosphatemicrickets and familial idiopathic LMW proteinuria with hypercalcinuria in Japanese patients.[21] Mutations in the CLCN5 gene encoding the electrogenic chloride/proton exchanger CIC-5 participating in the receptor-mediated endocytosis in the proximal tubule are a causative factor for Dent's disease type 1. Large number of different mutations in this gene was identified, until now, but no clear genotype-phenotype correlation was found. Dent's disease type 1 is characterized by symptoms exclusively related to proximal tubular dysfunction. Dent's disease of type 2 is thought to be a mild variant of oculocerebrorenal syndrome (Lowe syndrome) because both conditions are caused by mutation in the OCRL1 gene and therefore, the former is manifested sometimes with extrarenal features, including mild ocular involvement, mild intellectual disability, muscle hypotonia, umbilical hernia or short stature.[21,22]

Due to the mode of inheritance, Dent's disease affects primarily males. Female carriers remain predominantly asympto-matic, although they may have mild proteinuria and/or hypercalciuria.[22,23] The parents of our patient were cousins, and the mode of inheritance was not determined, due to lack of facility. The clinical features of Dent's disease are often subtle with the majority of patients being asymptomatic during infancy and early childhood. Initial symptoms may be variable and non-specific, including polyuria, proteinuria, microscopic haematuria or renal colic due to urolithiasis. Growth retardation may be present,[22]and that was observed in our patient. Proteinuria resulted from impaired reabsorption of proteins in proximal tubules is a typical and constant feature of Dent's disease and consists mainly of different LMW-proteins, including alpha-1- and beta-2-micro-globulin, cystatin C, lysozyme, retinol-binding protein (RBP), vitamin-D-binding protein and trace amounts of albumins.[22] Urinary protein excretion in patients with Dent's disease is usually moderate and only in rare cases of coexisting focal segmental

glomerulosclerosis reaches the nephrotic range.[19] Hypercalcinuria of different severity affects approximately 90% of patients. Urinary calcium excretion is usually higher in children than in adults, because calcinuria tapers with decreasing renal function.[22] Nephrocalcinosis is an important feature of Dent's disease affecting approximately 75% of patients.[22] It may serve as a cardinal feature for diagnosis. Nephrolithiasis is observed less commonly, and stones usually consist of calcium phosphate or calcium oxalate,[22]which was observed in our case. Hypercalciuria is thought to be a main aetiological factor of nephrocalcinosis and urolithiasis because urinary oxalate and citrate excretion is usually normal.We could not perform the 99mTc-DMSA renal scanning due to lack of facility in our hospital. In most children with Dent's disease, renal function is normal, but unfortunately, it declines during adulthood. The pathogenesis of this process is still unclear. But 30 to 80% of affected patients progress to end stage renal failure in the third to fifth decade of life.[23] The increased activation of PTH receptors on the apical membrane of the proximal tubule by excreted PTH may cause urinary phosphate loss, hypophosphatemia and rickets in a minority of patients.[24] Patients with Dent's disease show a poor accumulation of 99mTc-DMSA in renal parenchyma and rapid excretion of radiotracer due to proximal tubular endocytic dysfunction.[25] Some patients develop recurrent episodes of nocturnal blindness, probably due to renal loses of RBP. They are responsive to vitamin A therapy.[26] Currently, there is no definite strategy for the management of Dent's disease and recommended treatment will be mostly supportive. Thiazide diuretics and dietary salt restriction are used to reduce calciuria and to prevent the occurrence of nephrocalcinosis and nephrolithiasis. ACE inhibitors may be useful to reduce glomerular component of proteinuria. In recent animal studies a high-citrate diet seems to delay the progression of renal failure.[26] In future, more studies will be required for both diagnosis and management purpose.

Conclusion

In this study, we were confined with in the clinical features and conventional investigations. Most recent investigations such as genetic evaluation and renal biopsy will be required for final diagnosis. More studies are to be expected for further evaluation regarding Dent's disease.

References

1. Wrong OM, Nordern AGW, Feest TG. Dent's disease: a familial renal tubular syndrome with hypercalcinuria, tubular proteinuria, rickets, nephrocalcinosis and eventual renal failure. Q J Med. 1990;77: 1086–1087.

2. Lloyd SE, Pearce SHS, Fisher SE, Steinmeyer K, Schwappach B, Scheinman SJ et al. A common molecular basis for three inherited kidney stone diseases. Nature. 1996;379: 445–449.
3. Thakker RV. Pathogenesis of Dent's disease and related syndromes of X-linked nephrolithiasis. Kidney Int. 2000;57: 787–793.
4. Gunther W, Luchow A, Cluzeaud F, Vandewalle A, Jentsch TJ. ClC-5, the chloride channel mutated in Dent's disease, colocalizes with the proton pump in endocytotically active kidney cells. Proc Natl AcadSci USA. 1998;95: 8075–8080.
5. Devuyst O, Christie PT, Courtoy PJ, Beauwens R, Thakker RV. Intra-renal and sub cellular distribution of the human chloride channel, CLC-5 reveals a pathophysiological basis for Dent's disease. Hum Mol Genet. 1999;8: 247–257.
6. Hara-Chikuma M, Wang Y, Guggino SE, Guggino WB, Verkman AS. Impaired acidification in early endosomes of ClC-5 deficient proximal tubule. Biochem Biophys Res Commun. 2005; 329: 941–946.
7. Picollo A, Pusch M. Chloride/proton antiporter activity of mammalian CLC proteins ClC-4 and ClC-5. Nature. 2005;436: 420–423.
8. Scheel O, Zdebik AA, Lourdel S, Jentsch TJ. Voltage-dependent electro-genic chloride/ proton exchange by endosomal CLC proteins. Nature. 2005;436: 424–427.
9. Hoopes Jr RR, Shrimpton AE, Knohl SJ, Hueber P, Hoppe B, Matyus Jet al. Dent Disease with mutations in OCRL1. Am J Hum Genet. 2005;76: 260–267.
10. Shrimpton AE, Hoopes Jr RR, Knohl SJ, Hueber P, Reed AA, Christie PT et al. OCRL1 mutations in Dent 2 patients suggest a mechanism for phenotypic variability. Nephron Physiol. 2009;112: 27–36.
11. Cho HY, Lee BH, Choi HJ, Ha IS, Choi Y, Cheong HI. Renal manifestations of Dent disease and Lowe syndrome. Pediatr Nephrol. 2008;23: 243–249.
12. Sekine T, Nou K, Iyengar R, Xue Jun Fu, Matsuo M, Tanaka Ret al. OCRL1 mutations in patients with Dent disease phenotype in Japan. Pediatr Nephrol. 2007;22: 975–980.
13. Utsch B, Bo ¨kenkamp A, Benz MR, Besbas N, Dötsch J, Franke I et al. Novel OCRL1 mutations in patients with the phenotype of Dent disease. Am J Kidney Dis. 2006;48: e1– e14.
14. Sethi SK, Ludwig M, Kabra M, Hari P, Bagga A. Vitamin A responsive night blindness in Dent's disease. Pediatr Nephrol. 2009;24: 1765–1770.
15. Bogdanovic ´ R, Draaken M, Toroman-ovic ´ A, Dordevic ´ M, Stajic ´ N, Ludwig M: A novel CLCN5 mutation in a boy with Bartter-like syndrome and partial growth hormone deficiency. Pediatr Nephrol. 2010;25: 2363–2368.

16. Besbas N, Ozaltin F, Jeck N, Seyberth H, Ludwig M. CLCN5 mutation (R347X) associated with hypokalaemic metabolic alkalosis in a Turkish child: an unusual presentation of Dent's disease. Nephrol Dial Transplant. 2005;20: 1476–1479.
17. Sheffer-Babila S, Chandra M, Speiser PW. Growth hormone improves growth rate and preserves renal function in Dent disease. J Pediatr Endocrinol Metab. 2008;21: 279–286.
18. Copelovitch L, Nash MA, Kaplan BS. Hypothesis: Dent disease is an under recognized cause of focal glomerulosclerosis. Clin J Am Soc Nephrol. 2007;2: 914–918.
19. Frishberg Y, Dinour D, Belostotsky R, Becker-Cohen R, Rinat C, Feinstein S et al. Dent's disease manifesting as focal glomerulosclerosis: Is it the tip of the iceberg? Pediatr Nephrol. 2009;24: 2369–2373.
20. Hichri H, Rendu J, Monnier N, Coutton C, Dorseuil O, Poussou RV et al. From Lowe syndrome to Dent disease: correlations between mutations of the OCRL1 gene and clinical and biochemical phenotypes. Hum Mutat. 2011;32: 379–388.
21. Sahay M. Diseases of Renal Parenchyma. In Tech. Rijeka (Croatia): Dent's disease ; p.17-32.
22. Bockenhauer D, Bokenkamp A, Nuutinen M, Unwin Rvan't, Hoff W, Sirimanna T et al. Novel OCRL mutations in patients with Dent-2 disease. J Pediat Genet. 2012;1: 15-23.
23. Claverie-Martin F, Ramos-Trujillo E, Garcia Nieto V. Dent's disease: clinical features and molecular basis. Pediatr Nephrol. 2011;26(5): 693-704.
24. Amnigeri RA, Rajagopalan R. Hypophosphatemic rickets due to Dent's disease: A case report and review of literature. Indian J Nephrol. 2009;19(4): 163-166.
25. Weyer K, Nielsen R, Petersen SV, Christensen EI, Rehling M, Birm H. Renal Uptake of 99mTc-Dimercaptosuccinic Acid Is Dependent on Normal Proximal Tubule Receptor-Mediated Endocytosis. J Nucl Med. 2012;54(1): 1-7.
26. Becker-Cohen R, Rinat CH, Ben-Shalom E, Feinstein S, Ivgi H, Frishberg V. Vitamin A deficiency associated with urinary retinol binding protein wasting in Dent's disease. Pediatr Nephrol. 2012;27(7): 1097-1102.