Green Life Medical College Journal

Volume 2 Number 1 January 2017

Official Journal
of
Green Life Medical College

Website: greenlife.edu.bd

GREEN LIFE MEDICAL COLLEGE JOURNAL

Vol. 2, No. 1, January 2017

Journal Committee

Address of Correspondence
Editor, Green Life Medical College Journal
32, Bir Uttam K.M. Shafiullah Sarak, Dhanmondi, Dhaka-1205
Tel: 9612345-50 Ext. 1411
E-mail: ashraf.ahmeddrcme@gmail.com; Website: www.greenlife.edu.bd

ABOUT THE JOURNAL

Full Name of the Journal	:	Green Life Medical College Journal
Short Name	:	GMCJ
Nature of Publication	:	Bi-annual
Published From	:	Green Life Medical College
Address	:	32, Bir Uttam K.M. Shafiullah Sarak, Dhanmondi, Dhaka-1205 Tel: 9612345-50

AIMS & SCOPE:

The Green Life Medical College Journal is an English Language Scientific papers dealing with clinical medicine, basic sciences, epidemiology, diagnostic, therapeutics, public helath and healthcare in relation to concerned specialities. It is an official journal of Green Life Medical College and is published bi-annually.

The Green Life Medical College Journal of Bangladesh intends to publish the highest quality material on all aspects of medical science. It includes articles related to original research findings, technical evaluations and reviews. In addition, it provides readers opinion regarding the articles published in the journal.

INSTRUCTION TO AUTHORS:

Papers:

The Green Life Medical College Journal (published bi-annually) accepts contributions from all branches of medical science which include original articles, review articles, case reports, and letter to the Editor.

The articles submitted are accepted on the condition that they must not have been published in whole or in part in any other journal and are subject to editorial revision. The Editor preserves the right to make literary or other alterations which do not affect the substance of the contribution. It is a condition of acceptance that the copyright becomes vested in the journal and permission to republish must be obtained from the publisher. Authors must conform to the uniform requirements for manuscripts submitted to biomedical journals (JAMA 1997; 277: 927-34).

Legal Considerations:

Authors should avoid the use of names, initials and hospital numbers which may lead to recognition of a patient. A table or illustration that has been published elsewhere should be accompanied by a statement that permission for reproduction has been obtained from the authors or publishers.

Preparation of manuscript:

Each manuscript should indicate the title of the paper, and the name(s) and full address(es) of the author(s). Contributors should retain a copy in order to check proofs and in case of loss. Two hard copies of each manuscript (double-spaced) should be submitted. If a manuscript is accepted for publication in the GMCJ, the editor responsible for it may request a soft copy (a CD or via internet) of the final revision. Each paper will be reviewed for possible publication. The Editor may wish to see the raw data (electronic form) if necessary.

In preparing the manuscript, use double spacing throughout, including title, abstract, text, acknowledgement, references, table and legends for illustrations and font size 'Times New Roman 12'. Begin each of the following sections on a separate paper. Number pages consecutively.

The standard layout of a manuscript:

- Title page
- Abstract, including Keywords
- Introduction
- Methods
- Results
- Discussion
- Acknowledgements
- Funding
- List of references
- Tables & Figures
- Illustrations

The pages should be numbered in the bottom right-hand corner, the title page being page one, etc. Start each section on a separate page.

Title page:

A separate page which includes the title of the paper. Titles should be as short and concise as possible (containing not more than 50 characters). Titles should provide a

reasonable indication of the contents of the paper. This is important as some search engines use the title for searches. Titles in the form of a question, such as 'Is drinking frequent coffee a cause of pancreatic carcinoma?" may be acceptable.

The title page should include the name(s) and address(es) of all author(s). Details of the authors' qualifications and post (e.g., professor, consultant) are also required. An author's present address, if it differs from that at which the work was carried out, or special instructions concerning the address for correspondence, should be given as a footnote on the title page and referenced at the appropriate place in the author list by superscript numbers (1 *2* 3 etc.) If the address to which proofs should be sent is not that of the first author, clear instructions should be given in a covering note, not on the title page.

Abstract:

The 'Abstract' will be printed at the beginning of the paper. It should be on a separate sheet, in structured format (Introduction/Background; Methods; Results; and Conclusions) for all Clinical Investigations and Laboratory Investigations. For Reviews and Case Reports, the abstract should not be structured. The Abstract should give a succinct account of the study or contents within 350 words. The results section should contain data. It is important that the results and conclusion given in the 'Abstract' are the same as in the whole article. References are not included in this section.

Keywords:

Three to six keywords should be included on the summary page under the heading Keywords. They should appear in alphabetical order and must be written in United Kingdom English spelling.

Introduction:

The recommended structure for this section is

- Background to the study/Introduction
- What is known/unknown about it
- What -research question/hypothesis you are interested in
- What objective(s) you are going to address

The introduction to a paper should not require more than about 300 words and have a maximum of 1.5 pages double-spaced. The introduction should give a concise account of the background of the problem and the object of the investigation. It should state what is known of the problem to be studied at the time the study was started. Previous work should be quoted here but only if it has direct bearing on the present problem. The final paragraph should clearly state the primary and, if applicable, secondary aims of the study.

Methods:

The title of this section should be 'Methods' - neither 'Materials and methods' nor Patients and methods'. The Methods section should give a clear but concise description of the process of the study. Subjects covered in this section should include:

- Ethics approval/license
- Patient population
- Inclusion/exclusion criteria
- Conduct of the study
- Data handling
- Statistics
- Cognitive Task Analysis (CTA)

Ethical clearance:

Regardless of the country of origin, all clinical investigators describing human research must abide by the Ethical Principles for Medical Research Involving Human Subjects outlined in the Declaration of Helsinki, and adopted in October 2000 by the World Medical Association. This document can be found at: http://ohsr.od.nih.gov/guidelines/helsinki.html. Investigators are encouraged to read and follow the Declaration of Helsinki. Clinical studies that do not meet the Declaration of Helsinki criteria will be denied peer review. If any published research is subsequently found to be non-compliant to Declaration of Helsinki, it will be withdrawn or retracted. On the basis of the Declaration of Helsinki, the Green Life Medical Journal requires that all manuscripts reporting clinical research state in the first paragraph of the 'Methods' section that:

- The study was approved by the appropriate Ethical Authority or Committee.
- Written informed consent was obtained from all subjects, a legal surrogate, or the parents or legal guardians for minor subjects.

Human subjects should not be identifiable. Do not disclose patients' names, initials, hospital numbers, dates of birth or other protected healthcare information. If photographs of persons are to be used, either take permission from the person concerned or make the picture unidentifiable. Each figure should have a label pasted on its back indicating name of the author at the top of the figure. Keep copies of

ethics approval and written informed consents. In unusual circumstances the editors may request blinded copies of these documents to address questions about ethics approval and study conduct.

The methods must be described in sufficient detail to allow the investigation to be interpreted, and repeated if necessary, by the reader. Previously documented standard methods need not be stated in detail, but appropriate reference to the original should be cited. However, any modification of previously published methods should be described and reference given. Where the programme of research is complex such as might occur in a neurological study in animals, it may be preferable to provide a table or figure to illustrate the plan of the experiment, thus avoiding a lengthy explanation. In longitudinal studies (case-control and cohort) exposure and outcome should be defined in measurable terms. Any variables, used in the study, which do not have universal definition should be operationalised (described in such terms so that it lends itself to uniform measurement). Where measurements are made, an indication of the error of the method in the hands of the author should be given. The name of the manufacturer of instruments used for measurement should be given with an appropriate catalogue number or instrument identification (e.g. Keyence VHX-6000 digital microscope). The manufacturer's town and country must be provided, in the case of solutions for laboratory use, the methods of preparation and precise concentration should be stated.

Single case reports:
Single case reports of outstanding interest or clinical relevance, short technical notes and brief investigative studies are welcomed. However, length must not exceed 1500 words including an unstructured abstract of less than 200 words. The number of figures/tables must not be more than 4 and references more than 25.

Animal studies:
In the case of animal studies, it is the responsibility of the author to satisfy the board that no unnecessary suffering has been inflicted on the animal concerned. Therefore, studies that involve the use of animals must clearly indicate that ethical approval was obtained and state the Home Office License number or local equivalent.

Drugs:
When a drug is first mentioned, it should be given by the international non-proprietary name, followed by the chemical formula in parentheses if the structure is not-well known, and, if relevant, by the proprietary name with an initial capital letter. Dose and duration of the drug should be mentioned in sufficient details. If the drug is already in use (licensed by appropriate licensing authority), generic name of the drugs should preferably be used followed by proprietary name in brackets.

Present the result in sequence in the text, table and figures. Do not repeat all the data in the tables and/or figures in the text. Summarize the salient points. Mention the statistics used for statistical analysis as footnote under the tables or figures. Figures should be professionally drawn. Illustration can be photographed (Black and White glossy prints) and numbered.

Discussion:
Comments on the observation of the study and the conclusion derived from it. Do not repeat the data in detail, already given in the results. Give implications of the findings, their strengths and limitations in comparison to other relevant studies. Avoid un-qualified statements and conclusions which are not supported by the data. Avoid claiming priority. New hypothesis or implications of the study may be labeled as recommendations. Letters are welcome. They should be typed double-spaced on side of the paper in duplicate.

References:
References should be written in Vancouver style, numbered with arabic numerals in the order they appear in the text. The reference list should include all information, except for references with more than six authors, in which case give the first six names followed by et al.

Examples of correct forms of references:
Dorababu M, Prabha T, Priyambada S, Agrawal VK, Aryaa NC, Goel RK. Effect of Azadirachta indica on gastric ulceration and healing of bacopa monnierang in experimental NIDDM rats. *Indian J Exp. Biol 2004; 42: 389-397.*

Chapter in a book:
Hull CJ. Opioid infusions for the management of postoperative pain. In: Smith G, Covino BG, eds. Acute Pain. London: Butterworths. 1985,1 55-79.

Authors can write 'Letter to Editor' for any query.

All manuscripts for publication should be addressed to the Editor.

Professor M.A. Azhar
Principal
Green Life Medical College and
Editor-in-chief
Green Life Medical College Journal

ABOUT THE COLLEGE

INTRODUCTION

In 2005, about fifty distinguished physicians of the country started a hospital to give specialized care in the private sector. They named it Green Life Hospital and it turned out to be a great success. So in 2009, they decided to establish a medical college which will be a non-government, non-profit, self-financing project and will serve the humanity.

This College came into existence in 2009. The college commences its activities with the enrollment of 51 students in the 1st batch in 2010. Since inception, the college has undergone tremendous development and became a splendid centre for learning and development. At present we are enrolling 110 students each year. Among them, numbers of seats are reserved for overseas students.

We continue to evaluate and improve our programme to ensure the best medical education for the students. Our educational strategy is to create a conducive learning environment and to steer our students to acquire adequate knowledge, skills and temperament to practice medicine and be a competent health care professional group.

Green Life Medical College (GMC) is approved by the Ministry of Health and Family Welfare (MOHFW), Government of Bangladesh and Bangladesh Medical and Dental Council (BMDC) and affiliated to the University of Dhaka.

AIMS AND OBJECTIVES OF THE COLLEGE

Aims:

To create a diverse and vibrant graduate scholars in medical discipline and to create highly competent and committed physicians for the country.

Objectives:

- To provide an appropriate learning environment where medical students can acquire a sound theoretical knowledge and practical skills with empathetic attitude to the people.
- To carry out research in medical sciences to scale up the standard of medical education in the country.

LOCATION

The campus is located at 32, Bir Uttom K. M. Shafiullah Sarak (Green Road), Dhanmondi, Dhaka. The location is at the heart of the mega city Dhaka and is facilitated with very good communication networks.

The Medical College and the Hospital complexes have been raised in a multistoried fully air-conditioned building with an arrangement of approximately 500 patients. The building is equipped with state-of-the-art infrastructure, excellent with an out-patient department and adequate in-patient facilities.

GREEN LIFE MEDICAL COLLEGE JOURNAL

CONTENTS

Vol. 2 **No. 1** **January 2017**

EDITORIAL

Evidence Based Medicine (EBM) – New Approach to Teaching and Practice of Medicine

Evidence-based medicine (EBM) is the integration of best research evidence with clinical expertise and patient values.[1]

It represents integration of clinical expertise, patient's values and best available evidence in process of decision making related to patients health care. In addition, evidence based medicine is the conscientious, explicit, judicious and reasonable use of current best evidence in making decisions about the care of individual patients.

The practice of evidence based medicine is a process of lifelong, self-directed, problem-based learning in which caring for one's own patients creates the need for clinically important information about diagnosis, prognosis, therapy and other clinical and health care issues.[2]

The clinical evidence can be obtained by meta-analysis of several randomized controlled research (RCR), or from well designed controlled research; from comparative research, case study, from experts and clinical practice.

Evidence - based medicine when applied to medical education, advocates to the greatest extent possible, decisions and policies should be based on evidence, not just the beliefs of practitioners, experts, or administrators. It is supplemented with all available knowledge from the scientific literature so that best practice can be determined and applied. It promotes the use of formal, explicit methods to analyze evidence and makes it available to decision makers. It promotes programs to teach the methods to medical students, practitioners, and policy makers.[3]

Evidence-based medicine de-emphasizes intuition, unsystematic clinical experience, and pathophysiologic rationale as sufficient grounds for clinical decision-making, and stresses the examination of evidence from clinical research. It requires new skills of the physician, including efficient literature searching, and the application of formal rules of evidence in evaluating the clinical literature.

An important goal in teaching program is to educate physicians in the practice of evidence-based medicine. To do this some strategies should be included, like a weekly program for learning the necessary skills; sharing among faculty of approaches to teaching evidence-based medicine; and providing faculty with feedback on their performance as role models and teachers of evidence-based medicine. The influence of evidence based medicine on clinical practice and medical education is increasing.[4]

A key implication of the clinical decisions, recommendations, and practice guidelines must not only attend to the best available evidence, but also to the values and preferences of the informed patient. Values and preferences refer not only the patients' perspectives, beliefs, expectations, and goals for life and health, but also the processes individuals use to consider the available options and their relative benefits, harms, costs, and inconveniences. [5]

In practicing the evidence based medicine (EBM), the patient value is an important component. Therefore, the first National Health Service Constitution in Great Britain suggests that patient participation in decision making is a patient's right [6]. Also, in the United States, the Institute of Medicine designated evidence-based patient-centered health care delivery as a key feature of high-quality medical care.[7]

Therefore, EBM could be practiced in all the medical institutes for developing clinical expertise or clinical judgment and emphasis on patient values, preferences and improved patient's outcome. Also it might help in developing skills on retrieval of relevant scientific evidence and conducting research.

Journal of Green Life Med. Col. 2017; 2(1): 1-2

Prof. Dr. Ashraf Uddin Ahmed

Editor
Journal of Green Life Medical College
Head, Department of Community Medicine;
Chairperson Medical Education Unit (MEU) & Medical Skill Centre, Green Life Medical College

References:

1. Sackett DL, Rosenberg WM, Gray JA, Haynes RB, Richardson WS. Evidence based medicine: what it is and what it isn't. Br Med J 1996; 312:71-2.

2. zet Masic, Milan Miokovic, and Belma Muhamedagic . Acta Inform Med. 2008; 16(4): 219–225.

3. Sackett DL, Richardson WS, Rosenberg W, Haynes RB. Evidence-based medicine: how to practice and teach. 2. ed. Edinburgh: Churchill-Livingstone, 2000.

4. Gordon Guyatt, MD, MSc; John Cairns, MD; et.all. Evidence-Based Medicine A New Approach to Teaching the Practice of Medicine. JAMA. 1992; 268(17):2420-2425. doi:10.1001/jama.1992.03490170092032.

5. Guyatt GH, Haynes RB, Jaeschke RZ, et al. Users' Guides to the Medical Literature: XXV: evidence-based medicine: principles for applying the Users' Guides to patient care: Evidence-Based Medicine Working Group. *JAMA*. 2000; 284(10):1290-1296

6. UK Department of Health. National Health Services Constitution. http://www. dh.gov.uk/en/ Publications and statistics/ Publications/ PublicationsPo, Accessed June, 2016

7. Committee on Quality of Health Care in America, Institute of Medicine. *Crossing the Quality Chasm: A New Health System for the 21st Century.* Washington, DC: National Academic Press; 2001

ORIGINAL ARTICLE

Haematopoiesis Enhancing Effect of *Ipomoea Batatas* (Sweet Potato) Leaf Extract in Rats

HASAN MJ[1], ZABIR SM[2], ISLAM S[3], EVA EO[4]

Abstract

Introduction: *Blood cell count may be reduced in many clinical settings. Haematopoietic stimulants are needed to increase blood cell count. The purpose of the study was to investigate haematopoiesis enhancing effect of aqueous extract (AE) of Ipomoea batatas (sweet potato) leaf in rats.*

Methods: *Aqueous extract of sweet potato leaf was tested for its effect on blood cell count of rats. Rats were randomly divided into 4 groups. Group A served as control. Sweet potato leaf extract was administered orally as for Group B: 100 mg/Kg BW/day, Group C: 300 mg/Kg BW/day & Group D: 500 mg/Kg BW/day for 14 days. Blood samples were collected and then analyzed for RBC count, TC & DC of WBC and Platelet count. Statistical analysis of collected data was done by ANOVA (F test) & Student's unpaired t test. P value < 0.05 was considered as statistically significant.*

Results: *After 2 weeks, Group B showed slight but statistically non-significant (P value > 0.05) rise of blood cell count; Group C showed statistically significant (P value < 0.05) increment of all of 3 series of blood cells; Group D showed statistically highly significant (P value < 0.01) rise of total count of all of 3 series of blood cells. Differential Count of WBC study revealed increased distribution of neutrophil (maximum in Group D) with relative reduced distribution of lymphocyte.*

Conclusion: *Aqueous extract of sweet potato leaf has dose dependent haematopoiesis enhancing effect in rats.*

Key words: *Haematopoiesis, Myeloid stem cell, Bone marrow, Aqueous extract, Sweet potato leaf*

Journal of Green Life Med. Col. 2017; 2(1): 3-7

Introduction:

Blood cells (RBCs, WBCs & Platelets) are constantly formed by haematopoiesis within bone marrow. Suppression of bone marrow causes impaired haematopoiesis resulting in reduction of blood cell count. Haematopoiesis may be impaired and consequently anaemia, leucopenia, thrombocytopenia, bicytopenia or pancytopenia may occur in many clinical settings.[1]

Important Causes of Impaired haematopoesis leading to Pancytopenia are a) Marrow suppression by Anticancer drugs (Methotrexate, Cyclophosphamide), Immuno-suppressive drugs (Azathioprine, Cyclosporine), Antirheumatic drugs (Phenylbutazone, Gold, Penicillamine), Antimicrobial drugs (chloramphenicol, Sulphonamides), Antithyroid drugs (Carbimazole, Propylthiouracil), b) Marrow suppression due to Radiation/Radiotherapy; c) Marrow infiltration or replacement due to Leukaemia, Lymphoma, Multiple Myeloma, Metastatic carcinoma; d) Marrow hypoplasia like Aplastic anaemia; e) Overwhelming infections like Kala azar, TB, AIDS; f) Megaloblastosis due to vitamin B_{12} &/or Folic acid deficiency.[1,2] Bone marrow suppression followed by pancytopenia may result in many serious clinical consequences. Anaemia causes reduced oxygen carrying capacity of blood. Leucopenia predisposes to various viral, fungal, bacterial infections & even sepsis due to lack of body defense. Thrombocytopenia leads to defect in haemostasis followed by bleeding manifestations. So, any blood cell deficiency disorder is to be treated energetically.[1,2] Sometimes it is

1. Dr. Md. Jiaul Hasan, Assistant Professor, Department of Pharmacology, Manikganj Medical College, Manikganj
2. Dr. Sharif Mohammad Zabir, Lecturer, Department of Pharmacology, Dhaka Medical College, Dhaka
3. Dr. Md. Syful Islam, Lecturer, Department of Pharmacology, Shaheed M. Monsur Ali Medical College, Sirajganj
4. Dr. Eliza Omar Eva, Associate Professor, Department of Pharmacology, Dhaka Medical College, Dhaka

Address of Correspondence: Dr. Md. Jiaul Hasan, Assistant Professor, Department of Pharmacology, Manikganj Medical College, Manikganj

needed to stimulate bone marrow haematopoiesis actively. Different haematopoietic growth factors are now used therapeutically to increase blood cell count. ESAs and CSFs are commonly used as bone marrow modulators to treat toxicities of anticancer chemotherapy.[3] Erythropoietins stimulate erythropoiesis and combat severe anaemia due to anticancer therapy, CRF etc. Erythropoietins are very expensive. Their common adverse effects are hypertension, thrombotic events (Stroke, MI), worsening heart failure & allergic reactions. Moreover they are administered parenterally. Filgrastim, Lenograstim, Pegfilgrastim and Sargramostim are the CSFs that stimulate granulopoiesis. They are commonly used in treating cytotoxic therapy induced leucopenia (mostly neutropenia/agranulocytosis). To stimulate thrombopoiesis, Oprelkevin (recombinant IL-11) & Romiplostim (Thrombopoietin agonist) have also been developed.[4] These haematopoiesis stimulating drugs are costly, not easily available, and have potential adverse effects. Moreover their route of administration is not user friendly. This matter makes treatment of of pancytopenia more expensive & complicated. In Bangladesh & other developing countries, they are still used limitedly. So, development of cheap, easily available, easily usable & effective haematopoietic stimulants with fewer side effects is demanding.

Steroids are easily available, not so expensive and many of them are available in oral forms. Studies revealed variable stimulating effects of different steroid hormones on haematopoiesis. But still, steroids have not been established as haematopoietic stimulants due to their uncertain effect on bone marrow haematopoietic stem cell & widespread adverse effects.[5] As drugs of plant origin are cheap, easily available & often free from serious adverse effects, research to develop such effective drugs from plants is going on. Regarding this issue, some medicinal and herbal plants have been studied.

Ipomoea batatas (Sweet potato) is a creeping plant with perennial vines and adventitious roots, some of which produce swollen tubers. It is an ancient food from tropical America and the Pacific Islands. It is mainly cultivated from the tubers, used as vegetables, eaten boiled, baked, fried & grounded into flour to make biscuit, bread & other confectioneries. The leaf of sweet potato is used as food of domestic animals in Southeast Asia. It is rich in protein. It can also be used as an alternative for astringent, tonic, laxatives, fungicides. Sweet potato (*Ipomoea batatas*) leaf decoction is a folk remedy for asthma, bugbites, burns, fever, nausea, stomach distress and tumours. The leaf of Sweet potato is used to enhance the immune system of body. Also this plant has haematinic effects and has been used in the treatment of anaemia and other related ailments.[13]

Studies on effect of Sweet potato plant on some haematological parameters were carried out in different countries like Nigeria, Ghana & India. Some studies revealed that there was increment of total count of all of RBC, WBC & Platelet and increased distribution of neutrophil in experimental animals following administration of Sweet potato leaf's aqueous extract. These changes reflect enhancement of haematopoiesis due to stimulation of myeloid stem cells of bone marrow.[13,14] Though the plant is available in many districts of our country, study to detect haematopoietic effect of sweet potato leaf extract has yet not done in Bangladesh. This is why sweet potato leaf extract has been chosen for study. The aim of the work was to investigate the haematopoietic effect of aqueous extract of *Ipomoea batatas (*sweet potato) leaf in rats.

Methods:

The study was experimental one carried out in the Department of Pharmacology, Dhaka Medical College, Dhaka following approval from the ethical review committee, DMC, Dhaka. The sweet potato plants were collected and then taxonomically identified & authenticated by Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession number - 39543). The collected leaves of sweet potato plants were left to dry in air under shade in the dark at room temperature for 15 days. The air dried leaves were then converted into fine powder by electric grinding machine. The air dried powdered leaves were dissolved in distilled water in the ratio of 1:10 (w/v) in a round bottom flask with intermittent shaking for 24 hours to allow extraction. The liquid extract was then filtered and filtrate collected. Filtrates were then evaporated by vacuum rotary evaporator to convert into semisolid mass. The extract was kept in air tight glass container and stored at 4^{o} C until used. Before each use, extract was diluted in distilled water to obtain fresh preparation with desired concentration. The experiment was carried out on 32 albino rats, collected from ICDDRB, Dhaka. They were of either sex, weighing about 150-200 gm. Rats of different batches were kept in different metallic cages. They were allowed to feed on standard laboratory diet and to drink water ad libitum. Rats were randomly divided into 4 groups of each of 8 rats. Group A served as control that received distilled water 1 ml orally daily for 14 days. Group B received the extract 100 mg/Kg BW,

Group C received the extract 300 mg/Kg BW and Group D received the extract 500 mg/Kg BW orally daily for 14 days. At the end of 14 days, Blood sample was collected from each rat aseptically by sterile disposable syringe through cardiac puncture. Collected blood samples were then analyzed by Automated Haematology Analyzer SYSMEX XT-2000i & checked manually by microscopy of thin blood films.

Results:

Following laboratory analysis of blood samples, Data collection for each group of rats was done in a predesigned data collection sheet. Obtained data on Hb level, RBC count, TC & DC of WBC and Platelet count were recorded & compiled. Data were expressed as Mean +/- SD and tabulated & presented accordingly. Results of intervention groups were compared with that of control group. Statistical analysis of the recorded quantitative data was done by appropriate statistical tests, such as ANOVA (F test) & Student's unpaired t-test. Test result was considered as statistically significant at P value < 0.05. Obtained results & observed changes are expressed below.

Table-I

Total count of RBC, WBC & Platelet (as Mean +/- SD) of 4 Groups at the end of experiment

Variables	Group A	Group B	Group C	Group D
RBC count (x million/ccmm) (Mean +/-SD)	6.75 +/- 0.7	6.9 +/- 2.43	8.86 +/-0.52	10.9+/- 0.74
WBC count (x thousand/ccmm) (Mean +/-SD)	7.21 +/- 0.47	7.35 +/- 0.51	10.09 +/-0.72	17.23 +/- 1.65
Platelet count (x lac/ccmm) (Mean +/-SD)	2.25 +/- 0.93	2.55 +/- 0.75	4.60 +/- 0.88	7.60 +/- 1.13

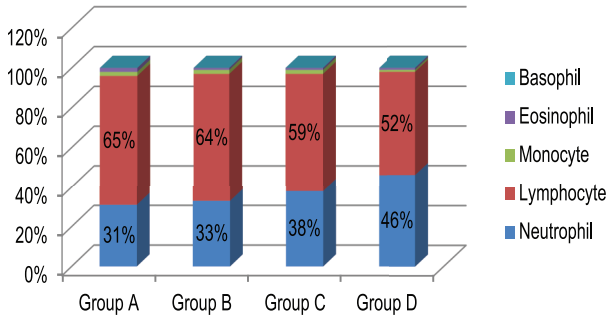


Fig.-1: *Component Bar Diagrams showing DC of WBC of 4 Groups*

Table-II

DC of WBC of 4 Groups at the end of experiment

Neutrophil (%)	31	33	38	46
Lymphocyte(%)	65	64	59	52
Monocyte (%)	2	2	2	1
Eosinophil (%)	2	1	1	1
Basophil (%)	0	0	0	0

Statistical analysis:

Mean, SD, Variance & SE of the relevant variables were calculated. Within group variance & between group variance were calculated to perform ANOVA (F test). Difference among 4 groups was analyzed by ANOVA (F test). Student's Unpaired t test was performed to compare between Group A & Group B, between Group A & Group C and between Group A & Group D. Difference between Group A & Group B was not significant statistically (P value > 0.05) (Table III). Statistically significant difference (P value < 0.05) was observed between Group A & Group C and between Group A & Group D. (Table IV & Table V).

Table-III

Comparison between Group A (control) & Group B (received extract 100 mg/Kg BW)

Variables	Group A	Group B	P value
RBC count (x million/cc mm) (Mean +/-SD)	6.75 +/- 0.7	6.9 +/- 2.43	> 0.05
WBC count (x thousand/ccmm) (Mean +/-SD)	7.21+/- 0.47	7.35+/- 0.51	> 0.05
Platelet count (x lac/cc mm) (Mean +/-SD)	2.25+/- 0.93	2.55+/- 0.75	> 0.05

Table-IV

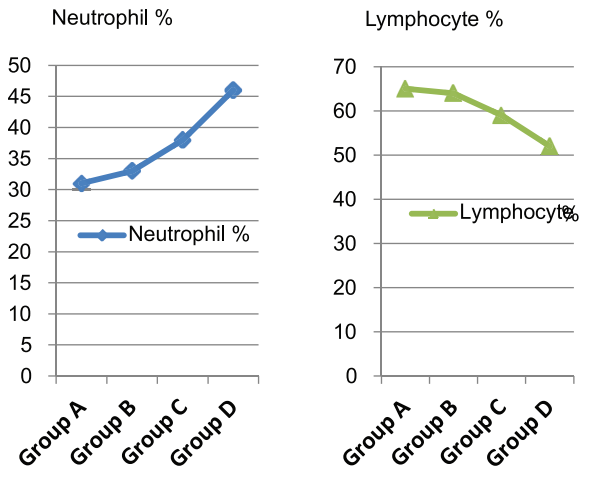
Comparison between Group A (control) & Group C (received AE of SPL 300 mg/Kg BW daily for 2 weeks)

Variables	Group A	Group C	P value
RBC count (x million/cc mm) (Mean +/-SD)	6.75 +/- 0.7	8.86 +/-0.52	< 0.05
WBC count (x thousand/ccmm) (Mean +/-SD)	7.21 +/- 0.47	10.09 +/-0.72	< 0.05
Platelet count (x lac/cc mm) (Mean +/-SD)	2.25 +/- 0.93	4.60 +/- 0.88	< 0.05

Table-V

Comparison between Group A (control) & Group D (received AESPL 500mg/Kg BW daily for 2 weeks):

Variables	Group A	Group D	P value
RBC count (x million/cc mm) (Mean +/-SD)	6.75 +/- 0.7	10.9+/- 0.74	< 0.01
WBC count (x thousand/cc mm) (Mean +/-SD)	7.21 +/- 0.47	17.23 +/- 1.65	< 0.001
Platelet count (x lac/cc mm) (Mean +/-SD)	2.25 +/- 0.93	7.60 +/- 1.13	< 0.001



(a) (b)

Fig.-2(a&b): *Line diagrams showing change of DC of WBC (Neutrophil & Lymphocyte)*

Discussion:

Effect of aqueous extract of *Ipomoea batatas* (Sweet potato) leaf on blood cell count of rats has been investigated in this study. It has been defined on some extent the role of the extract on bone marrow haematopoiesis to increase blood cell count. Study was carried out on 32 albino rats in the Department of Pharmacology, DMC, Dhaka. They were divided randomly into 4 groups of each of 8 rats. Out of 32 rats, Group A (n=8) received distilled water serving as control; Group B (n=8) received sweet potato leaf extract 100 mg/Kg BW, Group C (n=8) 300 mg/Kg BW & Group D (n=8) 500 mg/Kg BW all orally daily for 14 days. Duration of the study was selected on the basis of duration of the study done by Osime E.O, Ediale G.E, (2008).[7] Dose of extract including route of administration used in the study was selected according to dose & route used in the studies done by Osime E.O, Ediale G.E, (2008)[7] and G.A. Koffuor, P.E. Dadzeasah, (2012).[14] Before giving intervention, measured parameters of both control group & test groups were within reference range. After 14 days' administration of the extract at the dose of 100 mg/Kg BW, Group B showed slightly increased blood cell count, but not statistically significant (p value > 0.05). So, the extract at the dose of 100 mg/Kg BW was not able to exert significant effect on blood cell count of rats. Group C after receiving the extract at the dose of 300 mg/Kg BW for 14 days showed increased count of all of 3 series of blood cells. Statistical analysis revealed P value < 0.05. So, the difference was statistically significant. Group D that received the extract at the dose of 500 mg/Kg BW for 14 days showed marked increased count of all of 3 series of blood cells. Statistical analysis revealed P value < 0.01. So, the difference was statistically highly significant. It was observed that increment of blood cell count was dose dependent. Differential Count of WBC study revealed that there was increased distribution of neutrophil (maximum in Group D) with relative reduced distribution of lymphocyte.

As RBCs, Neutrophils & platelets are all formed from Common Myeloid Progenitors or Committed Myeloid stem cells, increment of total count of RBC, WBC, Platelet along with increased distribution of neutrophil indicates stimulation of the Myeloid stem cells by sweet potato leaf extract. So, the changes are suggestive of dose dependent increment of blood cell count due to enhanced bone marrow haematopoiesis with stimulation of myeloid stem cells of bone marrow by the extract. (Figure 3).

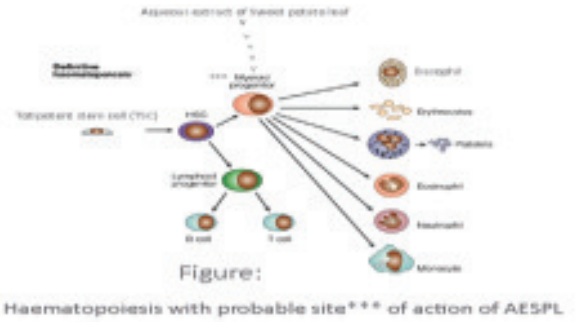


Fig.-3: *Haematopoiesis with probable site of action of Sweet potato leaf extract*

The enhancement of haematopoiesis was observed in this study with administration of the extract at the doses of 300 mg/Kg & 500 mg/Kg BW daily for 14 days. The enhanced haematopoietic activity with resultant increased blood cell count observed in this study is in well agreement with the work of Osime E.O. & Ediale G.E. (2008).[7] Probable mechanism of enhancement of haematopoietic activity might be direct or indirect effect of some phytochemical components of sweet potato leaf extract. Glycosides & Saponins present in the extract might stimulate directly or indirectly haematopoietic stem cells to proliferate & differentiate into blood cells. Tannin component of the extract by antioxidant property might protect stem cells & formed blood cells from free radical induced injury. Alkaloid of the extract might be responsible for restoration of haematopoiesis. Nutritional components of sweet potato leaves such as Iron, Folate, Vitamin B_{12}, Protein/Amino acids, Copper, Zinc, Manganese etc. might also support haematopoietic activity to some extent.

Conclusion:

Aqueous extact of *Ipomoea batatas* (Sweet potato) leaf has dose dependent haematopoiesis enhancing effect in rats. The enhanced haematopoiesis may be its direct or indirect stimulating effect on myeloid stem cells of bone marrow. Further study may be carried out on induced bone marrow suppressed animals of a large sample comparing with established haematopoietic stimulants. However, sweet potato leaf or leaf extract may be used in blood cell deficit conditions such as anaemia, leucopenia neutropenia, thrombocytopenia, bicytopenia or pancytopenia after ascertaining its safety in human.

References:

1. Watson HG, Craig JIO, Manson LM, Blood disease, Chapter 24, Davidson's Principles & Practice of Medicine, 22nd Edition, Churchill Livingstone Elsevier (2014), pp 991- 997, 1008, 1048
2. Firkin F, Chesterman C, Penington D & Rush B, Pancytopenia; Aplastic Anaemia, Chapter 6, de Gruchy's Clinical Haematology in Medical Practice, 5th Edition, Blackwell Science (2002), pp119-123
3. Brown CH, Bone Marrow Modulators: Colony-Stimulating Factors and Erythrocyte-Stimulating Agents, US Blood Journal, (2013), Vol 38 (1), pp 3-7
4. Masters SB, Agents used in Anaemia; Haematopoietic Growth factors, Chapter 33, Bertram G. Katzung's Basic and Clinical Pharmacology, 12th Edition, Mc Graw Hill LANGE; (2012), pp581-582, 590-595
5. Edward H, Reisner JR, Tissue Culture of Bone marrow: Effect of Steroid hormones on Haematopoiesis in vitro, American Soceity of Haematology Journal, (1966), Vol. 27 (4), pp 460-469.
6. Osime EO, Ediale GE, Study on Effect of Sweet potato (*Ipomoea batatas*) Leaf Extract On Some Haematological Parameters Using Rabbits, Journal of Medicine and Biomedical Research, (2008), Vol 7, pp 1-5.
7. Lohar PS, Lohar MS, Choudury SR, Erythropoietic effects of some medicinal plants of India on experimental rat model, Slovak Journal of Animal Science, (2009), Vol 42, pp 95-98.
8. Koffuor GA, Dadzeasah PE, Haematopoetic effect of an ethanolic leaf extract of Ipomoea involucrata P. Beauv in phlebotomized Newzealand White Rabbits, Journal of Medical and Biomedical Sciences, (2012), V0l 1(2), pp 10-16.
9. Mbaeyi-Nwaoha IE, Emejulu VN, Evaluation of phytochemical composition and antimicrobial activity of Sweet potato *(Ipomoea batatas*) leaf, Pakistan Journal of Nutrition, (2013), Vol 12(6), pp 575-586.
10. Islam S, Sweet potato (*Ipomoea batatas* L.) Leaf: Its Potential Effect on Human Health and Nutrition, Journal of Food Science, (2006), Vol 71(2), pp 13–121.

ORIGINAL ARTICLE

Effect of Ethanol Extract of *Psidium Guajava* Linn Leaves on Blood Glucose Level in Normal and Experimental Diabetic Rats

MOMTAZ A[1], SHARMIN K[2], RAHMAN S[3], SULTANA N[4], IMON AA[5]

Abstract

Introduction:* Psidium guajava *Linn. (guava) is used not only as food but also as folk medicine in subtropical areas around the world because of its pharmacological activities. In particular, the leaf extract of guava has traditionally been used for treatment of diabetes in East Asia and other countries.

Objective: The study was done to evaluate the anti-diabetic effects of ethanol extract of* Psidium guajava *leaves in experimental rats.

Methods: The experiment was carried out in the department of Pharmacology of Dhaka Medical College, Dhaka, from July 2012 to June 2013. 24 healthy Long Evans Norwegian strains of rats were equally divided into four groups (A, B, C and D). Group A (control) received standard rat food. Group B was given ethanol extract of* Psidium Guajava *leaves 100mg/kg/day. Diabetes was introduced by administration of Alloxan 120/mg/kg body weight in group C and D. ethanol extract of* Psidium Guajava *leaves 100mg/kg/day was given to group D. Total duration of the experiment was 15 days.

Results: The rats receiving ethanol extracts of* Psidium guajava *leaves 100 mg/kg/day in group B produced no significant changes in blood glucose level as compared to control group A. Administration of 100mg/kg body weight of ethanol extract of* Psidium guajava *leaves in group D produced a significant (P<0.001) reduction of blood glucose level as compared to diabetic control group C.

The observation and results showed that ethanol extract of* Psidium guajava *leaves has no effect on blood glucose level in normal but can reduce blood glucose level in experimentally induced diabetic rats.

Key words: Diabetes mellitus,* Psidium guajava*, alloxan, rats, blood glucose.

Journal of Green Life Med. Col. 2017; 2(1): 8-11

Introduction:

Diabetes mellitus is one of the common metabolic disorders known to mankind, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders.[1] In modern medicine no satisfactory effective therapy is available to cure diabetes mellitus.[2] Herbal medicine have several advantages such as fewer side effects, better patient tolerance, relatively less expensive and well accepted due to long history of use. The more important cause is that herbal medicines provide rational means for the treatment of many diseases that are obstinate and incurable in other systems of medicine.[3]

Guava tree is basically from Meso Americam area. It can also find in tropical and subtropical areas. Guava tree is member or myrtaceae family, all the parts of this tree widely use in curing many health problems. A lot of work on pharmacological researches has been done to demonstrate the use of extract from guava leaves which proved that guava leaves extracts are such a useful medicine, widely used by doctors and pharmacists. WHO

1. Dr. Azmary Momtaz, MBBS, M Phil, Assistant Professor, Department of Pharmacology, Delta Medical College, Dhaka, Bangladesh
2. Dr. Khaleda Sharmin, MBBS, M Phil, Assistant Professor, Department of Pharmacology, University Dental College
3. Dr. Sharmin Rahman, MBBS, M Phil, Assistant Professor, Department of Pharmacology, Ibrahim Medical College, Dhaka, Bangladesh
4. Dr. Nashid Sultana, MBBS, M Phil, Assistant Professor, Department of Pharmacology, Delta Medical College
5. Dr. Ashik Abdullah Imon, BDS, M.S, Ex-Chief resident, Department of Oral and maxillofacial Surgery, BSMMU

Address of Correspondence: Dr. Azmary Momtaz, MBBS, M Phil, Assistant Professor, Department of Pharmacology, Delta Medical College, Dhaka, Bangladesh, Email: lizasomc@yahoo.com.

(world health organization) also says that plants would be the best source of obtaining different types of medicines and drugs. These natural products are widely used by human with its effective results. Extraction from guava leaves mostly essential oil, tannins, flavonoids, phenol compounds, carotenoids and vitamin C.[4]

The leaves of guava are rich in flavonoid, in particular, quercetin. Quercetin is the main flavonoid in guava leaves contribute to its anti-hyperglycemic effects. Guava leaves also has antioxidant properties which is attributed to the polyphenols found in the leaves[5]. It has also been demonstrated that flavonoids of guava leaves can act as insulin secratagogues or insulin mimetics, probably by influencing the pleiotropic mechanism, to attenuate the diabetic complications, besides, the drug candidates have been found to stimulate glucose uptake in peripheral tissues, and regulate the activity and/or expression the rate-limiting enzymes involving in carbohydrate metabolism pathway. In study it was found that flavonoids were act directly on pancreatic beta cells leading to activation of the cAMP/PKA signaling cascade to exert an insulinotropic effect.[6]

With this background information, in this study, attempt has been made to evaluate the effect of *Psidium Guajava* Linn. extract in normal and experimental diabetic rats. Alloxan has been chosen to induce DM in rats. Blood glucose level has been estimated to the extent of pancreatic damage.

Methods:
The study has been performed in the department of Pharmacology at Dhaka medical college, Dhaka from July 2012 to June 2013.

A total number of 24 healthy rats of Long Evans Norwegian strain weighing between 140-150 gm and age between 8-10 weeks which were purchased from Bangladesh Centre for Scientific and Industrial Research (BCSIR) Lab were used for the present study. The rats were allowed to live at room temperature with 12 hours of light and 12 hours dark schedule. They were fed normal rat diet and given water *ad libitum*.

The rats were divided into 4 groups (A, B, C and D). Each group consists of 6 rats.

Plant materials:
Psidium guajava leaves were collected from local garden. Leaves were dried under shade at room temperature less than 40 degree Celsius.

Preparation of extract:
Ethanol extract was made in the Drug Research Laboratory of Center for Advanced Research of Sciences (CARS) of Dhaka University. 1 kg of *Psidium guajava* (Guava) leaves were cleaned and shed dried. Then it was crushed into coarse powder and soaked in absolute ethanol (2L) with continuous shaking (40rmp) at 25°C for three days and filtered by filter paper. The ethanol extract was evaporated under vacuum rotator evaporator at 35 degree temperature to obtain final deep green semisolid extract. A total of 30 gram extract was found in this way.

Experiment Design:
The experiment was divided into two parts: Experiment-1 and Experiment-2.

Experiment-1:
This part of experiment was carried out to demonstrate the effect of ethanol extract of *Psidiam guajava* (guava) leaves on blood glucose level in experimental non diabetic rats. It was comprised of 12 rats which were divided into 2 groups, each having 6 rats. Groups were labeled as Group-A, Group-B. All the rats were fasted over night before collection of blood.

Group – A (control group)- was given standard rat food for 14 days. Fasting blood glucose was estimated on day 1 and day 15 of the experiment.

Group – B- was given ethanol extract of *Psidiam guajava* (guava) leaves 100mg/kg/day orally along with standard rat food for 14 days. Fasting blood glucose level was estimated on day 1 and day 15 of the experiment.

Experiment 2:
It was comprised of 12 rats which were divided into 2 groups each having 6 rats. Groups were labeled as Group-C and Group-D. All the rats were fasted overnight before collection of blood.

Group-C (Diabetic control group) was given alloxan 120mg/kg intraperitonially for induction of diabetes on day 1. After alloxan injection rats were given standard food. Fasting blood glucose level were estimated on day 1 (before alloxan), on day 4 on day 15 of the experiment.

Group-D was given alloxan 120mg/kg intraperitoneally on day 1. After alloxan injection rats were given standard food. Then after 3 days ethanol extract of *Psidiam guajava* (guava) leaves 100mg/kg/day was given orally along with standard food for 10 days. Fasting blood glucose level was estimated on day 1 (before alloxan), on day 4 and on day 15 of the experiment.

Results:
Effect of EEPGL (ethanol extract of *Psidium guajava* leaves) on blood glucose level in non-diabetic rats:

In group-A, the blood glucose levels (mean± SD) were 5.80± 0.51 and 5.80± 0.47 on day 1 and day 15 respectively. Percentage change was 2.30. The results are shown in table-1.

In group-B, the blood glucose levels (mean± SD) were 5.70± 0.39 and 5.60± 0.46 on day 1 and day 15 respectively. Percentage change was 2.19. The results are shown in table- I.

Table-I

Showing the effect of EEPGL (ethanol extract of Psidium guajava leaves) on blood glucose level in non-diabetic rats:

Group	FBG (mmol/L) on day 1 (Mean±SD)	FBG (mmol/L) on day 15 (Mean±SD)	Percent change
A (n=6)	5.80± 0.51	5.80± 0.47	+ 2.30
B (n=6)	5.70± 0.39	5.60± 0.46^{ns}	+ 2.19

Percent change on day 15 from day 1. Comparison of FBG level on day 15 with control (group-A) done by unpaired student's't' test.
ns= not significant.

Group A- Standard rat diet and water were given.

Group B- Ethanol extract of *Psidium guajava* leaves at 100mg/kg/day and standard rat diet and water were given.

Effect of alloxan on blood glucose level of group C and D rats on day 4:

In group C, the blood glucose levels (mean±SD) were 5.60±0.64 and 15.00 ± 3.38 on day 1 and day 4 respectively. Percentage change was 173.90.

In group D, the blood glucose levels (mean±SD) were 5.80±0.64 and 15.04 ± 3.46 on day 1 and day 4 respectively. Percentage change was 162.63.

All the results are shown in table II.

Table-II

Showing the effect of alloxan on blood glucose level of group Cand D rats on day 4.

Group	FBG (mmol/L) on day 1 (before alloxan) (Mean±SD)	FBG (mmol/L) on day 4 (after alloxan) (Mean±SD)	Percent change
C (n=6)	5.60±0.64	15.00±3.38	+173.90
D (n=6)	5.80±0.64	15.04±3.46^{ns}	+162.63

Percent change on day 4 from day 1. Comparison of FBG level on day 4 with control (group C) done by unpaired Student's't' test.

ns= not significant

Group C and D were given alloxan 120mg/Kg single i.p. injection on day 1.

Effect of EEGL (ethanol extract of *Psidium guajava* leaves) on blood glucose level in diabetic rats:

In group C, the blood glucose levels (mean±SD) were 5.50±0.64 and 16.00 ± 2.83 on day 1 and day 15 respectively. Percentage change was 192.38.

In group D, the blood glucose levels (mean±SD) were 5.80±0.64 and 8.03 ± 2.03 on day 1 and day 15 respectively. Percentage change was 41.50.

Table-III

Showing the effect of EEPGL (ethanol extract of Psidium guajava) leaves on blood glucose level in diabetic rats.

Group	FBG (mmol/L) on day 1 (Mean±SD)	FBG (mmol/L) on day 15 (Mean±SD)	Percent change
C (n=6)	5.50±0.64	16.00±2.83	+192.38
D (n=6)	5.80±0.64	8.03±2.03***	+41.50

Percent change on day 15 from day 1.

Comparison of FBG level on day 15 with control (group C) done by unpaired Student's't' test.

***= significant at P <0.001

Group C – Standard rat diet and water were given.

Group D – Ethanol extract of *Psidium guajava* leaves at 100mg/Kg/day and standard rat diet and water were given.

Discussion:

The present study was carried out to evaluate the effect of ethanol extract of *Psidium guajava* leaves on experimentally induced diabetes mellitus in rats. The blood glucose lowering effect of ethanol extract of *Psidium guajava* leaves was tested in non diabetic and experimentally induced diabetic rats. The ethanol extract of *Psidium guajava* leaves was given for 14 days in non diabetic rats and for 10days in alloxan induced diabetic rats.

In the present study, diabetes was induced by alloxan. The dose and route of administration of alloxan monohydrate was selected from Andrade *et al.* (2000)[7] and Kim *et al.* (2006)[8] respectively. The blood glucose levels in animals were measured 72 hours after administration of alloxan which was done according to experiment of Etuk *et al.* (2010)[9]. In this study, intraperitonial (ip) administration of single dose of alloxan (120mg/kg), increased blood glucose level significantly. Similar observations were reported by number of researchers. Ghosh *et al.* (2010)[10]

observed the condition of diabetes after 24 hours of intravenous injection of sterile, freshly prepared 1% alloxan monohydrate solution at a dose of 40mg/kg in albino rats. Jeloder *et al.* (2005)[11] in their experiment observed the effect of fenugreek, onion and garlic on blood glucose and histopathology of pancreas of diabetic rats, by inducing diabetes in 20 out of 25 adult male albino rats by intraperitonial injection of 185mg/kg of alloxan. In the present study, the rise of blood glucose level in experimental diabetic rats was also very high.

The dose of *Psidium guajava* leaves (100mg/kg body weight), and duration used in this study was selected in keeping conformity with the dose and duration used in research work by Yesmin (2010).[5]

The leaves of guava are rich in flavonoid, in particular, quercetin. Quercetin is the main flavonoid in guava leaves contribute to its anti hyperglycemic effects (Yesmin, 2010)[5]. Flavonoid compounds act against diabetes mellitus either through their capacity to avoid glucose absorption or to improve glucose tolerance (Bhathena *et al.* 2002).[12] The (-)-epicatechin gallate, myricetin, quercetin, apigenin, (-)- epigallocatechin gallate, and (-)-epigallocatechin demonstrated a marked reduction in glucose absorption, when compared with control, by competitive inhibition of sodium dependent glucose transporter (Shimiju *et al.* 2000).[13] It has also been demonstrated that flavonoid compounds of guava leaves can act as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanism, to attenuate the diabetic complications, besides, the drug candidates have been found to stimulate glucose uptake in peripheral tissues, and regulate the activity and/or expression the rate limiting enzymes involving in carbohydrate metabolism pathway. In study it was found that flavonoid were act directly on pancreatic beta cells leading to activation of the cAMP/ PKA signaling cascade to exert an insulinotropic effect (Liu *et al.* 2006).[6] As a result bioflavonoids are now a day regarded as promising and significantly attractive natural substance to enrich the current therapy option against diabetes.

It was observed that the ethanol extract of *Psidium guajava* leaves has glucose lowering effect in alloxan induced diabetic rats but no effect on blood glucose level on non diabetic rats. The result suggested that the ethanol extract of *Psidium guajava* leaves may be a useful anti diabetic agent in the treatment of diabetes mellitus.

It is suggested to measure plasma insulin level, haemoglobin A_1C, liver glycogen level, lipid hydroperoxidation level and free radical in the tissues after treatment with ethanol extract of *Psidium guajava* leaves. Despite all these limitations, interpretation of the results obtained in this study was made carefully and cautiously.

Acknowledgement:

The authors are grateful to the following persons- Dr. Md. Zakir Sultan, Senior scientist and Head, Drug Research Laboratory, Center for Advanced Research in Science, University of Dhaka for his cordial help, advice and very kind co operation for preparation of extract. Special thanks to Mr, Shihabul Alam, Medical technologist and Mr. Awlad Ali, caretaker of animal house of Dhaka Medical College for their contribution to complete the experiment.

References:

1. Ajao SM, Olayaki LA, Oshiba OJ, Jimoh RO, Jimoh SA, Olawepo A and Abioye AIR. Comparative study of the hypoglycemic effects of coconut water extract of P*icralimanitida* seeds (Apocynaceae) and Daonil in alloxan-induced,diabetic albino rats. African Journal of Biotechnology: 2009; 8(4): p. 574 -576.
2. Edem DO. Hypoglycemic effects of ethanolic extracts of alligator pear seed (*Perseaamericana* Mill) in rats. European Journal of Scientific Research: 2009; 33(4), p. 669-678.
3. Waqar MA, and Shaukat S. Diabetes Mellitus- The pertinent Exploration of Herbal Treatment, Jour Chem Soc Pak: 2006; 28(4): p. 391-396.
4. Porwal V, Sing P, Gurjar D. A Comparative Study on Different Methods of Extracrtion from Guajava Leaves for Curing Various Health Problems. Int J Engineering Research and application (IJERA). 2012; 2(6): 490-496.
5. Yesmin N. Hypoglycemic Effect of Guava Leaves Upon Alloxan Induced Diabetic Rats, Department of Pharmacology, MD, Our Lady Fatima University; 2009-2010.
6. Liu D, Zhen W, Yang Z, Carter JD, Si H, Reynolds KA. *Diabetes* **55**, 2006. 1043-1050.
7. Andrade SI, Monslave, MCR, Pena JEDL, Polanco AC, Palomino MA, Velasco AF. Streptozotocin and alloxan in experimental diabetes: comparison of the two models in rats. Acta Hischem Cytochem: (2000); 33(3): 201-208.
8. Kim JS, Ju JB, Choi CW, Kim SC. 'Hypoglycemic and antihyperlipidemic effect of Four Korean Medicinal Plants in Alloxan Induced diabetic rats. Am J Biochem & Biotech: 2006; 2(4): 154-160.
9. Etuk EU, Muhammad BJ. Evidence based analysis of chemical method of induction of diabetes mellitus in experimental rats. J Res Pharmachol Sci: 2010; 1(2):139-142.
10. Ghosh R, Shartchandra K, Rita S, Thokchom IS. Hypoglycemic activity of Ficus hispida (bark) in normal and diabetic albino rats. Indian J Pharmachol: 2004; 36(4):222-225.
11. Jeloder GA, Maleki M, Motadayen MH, Sirus S. Effect of Fenugreek, Onion and Garlic on Blood glucose and Histopathology of Pancreas of alloxan induced diabetic rats. Ind J Med sci*:* 2005; 59(2):64-69.
12. Bhathena SJ, Velásquez MT. Am J Clin Nutr: 2002; 76:1191-1201.
13. Shimizu M, Kobayashi Y, Suzuki M, Satsu H, Miyamoto Y. Bio Factors: 2000**;** 13: 61-65.

ORIGINAL ARTICLE

Accessibility and Availability of Family Planning Services and Antenatal Care Received During the Last Child Birth of the Women of Dhamrai Upazila

AHMED AU[1], CHOUDHURY S[2], SHARMIN T[3], AZIM E[4], KAMRUN S[5], KHAN S[6], KARIM F[7]

Abstract

Background: Over the years the contraceptive prevalence rate has increased in Bangladesh. The aim of this study was to assess the prevalence of using contraceptive methods and find out more about their knowledge and choices in contraceptive methods and to get the extent of antenatal care (ANC) services.

Methodology: A cross sectional descriptive study was conducted from 1st November to 28th February among 200 respondents with a semi- structured questionnaire by a face to face interview with them regarding their contraceptive practice and extend of ANC.

Results: Majority (55.5%) of the women used contraceptive pills and 11.6% used injectable contraceptive. Most of the women were house-wives. 41.3% women chose drug stores for purchasing of the contraceptive methods. Those who received the family planning methods from the health workers found them to be very friendly and approachable. About 42% women experienced the side effects of contraceptives and 40% spouse disliked it. These were some of the reasons why contraceptive methods were discontinued or not used at all. As far as antenatal care services were considered, people still did not realize the importance of healthy maternal and child health care. Two-third women (74.5%) said that they received ANC while they were pregnant.

Conclusion: The growth curve of the country is on the rise, which is an issue of concern. The reasons which have been posed to not use family planning methods can be understood and be dealt with those. This can only be achieved if existing barriers and availability are identified and addressed. By increasing awareness about the different contraceptive methods through health promotion and health education, people can make an informed choice close monitoring, planning and policy making can ensure pregnant women to receive ideal antenatal visits or at least three visits to reduce the maternal mortality ratio.

Key words: Accessibility, Availability, Family Planning Services, Contraceptive Methods, Ante Natal Care

Journal of Green Life Med. Col. 2017; 2(1): 12-16

1. Prof. Dr. Ashraf Uddin Ahmed, Professor and Head, Dept. of Community Medicine, Green Life Medical College
2. Dr. Shamima Choudhury, Lecturer, Dept. of Community Medicine, Green Life Medical College
3. Dr. Tanima Sharmin, Assistant Professor, Dept. of Community Medicine, Green Life Medical College
4. Dr. Ehsamul Azim, Associate Professor, Dept. of Community Medicine, Green Life Medical College
5. Dr. Sayma Kamrun, Lecturer, Dept. of Community Medicine, Green Life Medical College
6. Dr. Sheela Khan, Assistant Professor, Dept. of Community Medicine, Green Life Medical College
7. Dr. Farhana Karim, Lecturer, Dept. of Community Medicine, Green Life Medical College

Address of Correspondence: Prof. Dr. Ashraf Uddin Ahmed, Professor and Head, Dept. of Community Medicine, Green Life Medical College, Email: ashraf.ahmeddrcme@gmail.com

Background:

Bangladesh is one of the most densely populated countries in the world with population density of 1087 persons per square kilometer.[1,2] The growth rate has been brought down to 1.1 percent[3] and Bangladesh is in 3rd stage of demographic cycle.[4] This is undoubtedly a good step forward but a lot of work still remains to be done on the field of Family Planning. The use of contraception among married women in Bangladesh has increased gradually, from 8 percent in 1975 to 61 percent in 2011[5,6]. While it has helped in reducing the population growth, is still not quite enough and more awareness and health education is still needed to be done in this sector. Over the years the

contraceptive prevalence rate has increased in Bangladesh[7,8]. This study, embarked on this journey to interact with the people in rural areas and find out more about their knowledge, and choices in contraceptive methods. Antenatal care services were another area the present study wanted to know the extent to which the antenatal care has improved in the study area, especially considering the low socio economic conditions of rural areas.

Methods:

A cross sectional descriptive type of observational study was conducted in the village of Barigaon, Mamura, Maukhali, Boro Chondral & Chhoto Chondrial, to find the accessibility and availability of family planning methods and the proportion of receiving ANC during the last child birth of the respondent. The study was conducted among 200 women of reproductive age group with at least one child and age of last child being less than one year. Non probability purposive sampling was done to select the respondents. The period of study was from November, 2014 to February, 2015.

Semi- structured questionnaire was used to collect necessary data by a face to face interview by the interviewers. Before the data collection Questionnaires were pretested and revised. After collection, each questionnaire was sorted and cleaned for its consistency and comprehensiveness. Then cleaned data were analyzed through computer based software SPSS v. 16 and Microsoft Excel 2007.

Results:

Figure 1 shows that out of 200 respondents, majority were in the age group of 26-35yrs.

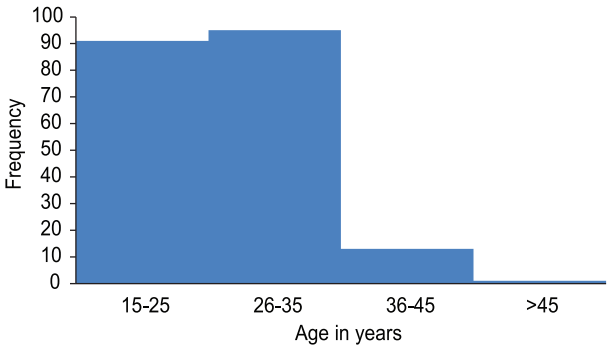


Fig.-1: *Histogram showing the age of the respondent (in completed years)*

Figure 2 shows that about 38% of the women completed secondary level of studies, 22.5% primary level, 11.5% SSC, 11% HSC & above; 9.5% can write their name only, and 7.5% with no education.

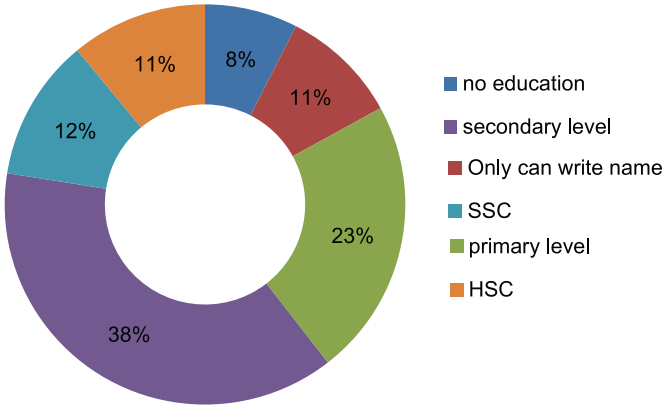


Fig.-2: *Educational status of respondents*

All most all (91.5%) had idea about contraception whereas only 17(8.5%) had no idea about contraception.

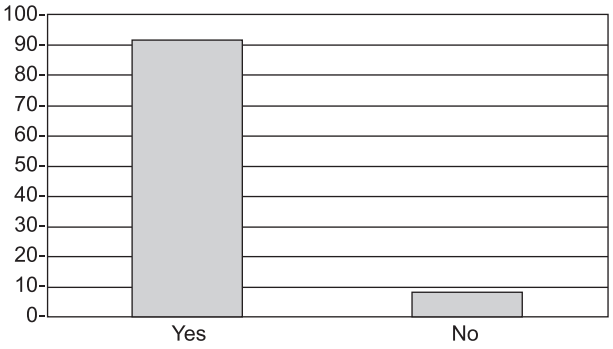


Fig.-3: *Idea about Family Planning methods among women*

Figure-4 shows that about two-third of women, (77.5%) are currently using contraceptives and only (22.5%) are not.

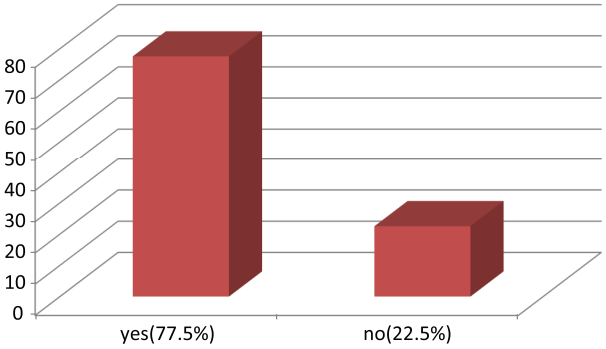


Fig.-4: *Distribution of women by currently using family planning methods*

Fig. 5 shows that 155 women the most common contraceptive method were pills 86(55.5%) , then injectable 18(11.6%), IUD 11(7.1%), Sterilization 5(3.2%) and followed abstinence/withdrawal 2(0.6%).

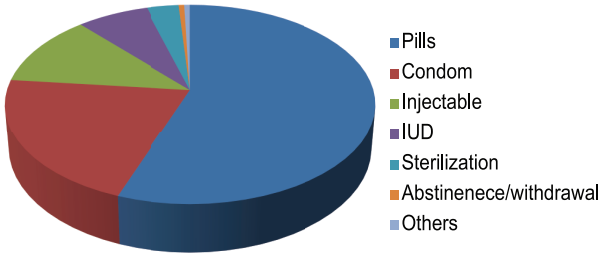


Fig-5: *Family Planning methods used by the respondents*

Fig. 6 shows that among 155 respondent currently using contraceptives, above half of them that is 65(41.93%) obtained it from drug store and 48 (30.96%) obtained from health workers, 40 (25.8%) from health center and very few 2(1.29%) from other sources.

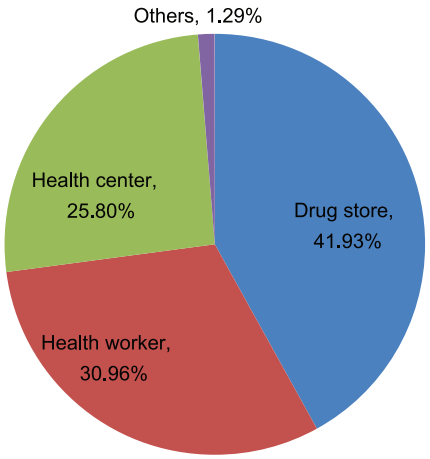


Fig.-6: *Places of obtaining contraceptive methods*

Table-I

Accessibility and availability of contraceptive methods in the drug store (n=65)

Factors	Category	n (%)
Accessibility of drug store	Not very far	50 (76.92%)
	Very far	5 (7%)
	Few minutes walking distance	5 (7%)
	Few minutes by vehicle	5 (7%)
Person who brought the contraceptive materials from drug store	Husband	56 (86.15%)
	Self	8 (12.3%)
	Others	1(1.5%)
Perception of price of contraceptive material	Expensive	11 (16.93%)
	Cheap	20 (30.77%)
	Reasonable	34 (52.3%)

Table-I shows that among 65 respondents obtaining family planning materials from drug store, maximum 50(76.92%) of them said that the drug store was not very far, 5(7.6%) said very far, 5(7.6%) said it tooks few minutes to walk and 5(7.6%) said it took few minutes by vehicle. Most of them 56(86.15%) said that their husband brought it, 8(12.3%) respondent brought it by themselves and for 1 respondent (1.5%) others brought it. Little above half of them 34(52.3%) of the users said that the price was reasonable, about one-forth of 20(30.77%) found it cheap and the rest 11(16.93%) found it expensive.

Table-II

Perception of the women who received FP methods from Health worker (n=48)

Factors	Category	n(%)
Regularity of materials provided by Health Workers	Regularly	95.8%
	Irregularly	4.2%
Friendly Behaviour of Health Worker	Friendly	95.8%
	Not friendly	4.2%

Table-II revealas among 48 respondents obtaining contraceptives from Health Workers(HW), most of them 46(95.8%) said they had received the materials from HW

Table-III

Perception about the availability of the service in the Health Center (n=40)

Factors	Category	n(%)
Availability of contraceptives in Health Center	Available	33 (82.5%)
	Not available	7 (17.5%)
Money charged for Family Planning method in Health Center	Money charged	13(32.5%)
	Money not charged	27 (67.5%)
Amount of money given by the users for Family	≤ 50 TK	5(38.47%)
Planning methods in health center (n=13)	51-100 TK	7(53.85%)
	≥100 TK	1(7.7%)

regularly and 2(4.2%) said, irregularly. Majority that is 46(95.8%) said that the Health worker were friendly whereas very few said that the Health worker were not friendly.

Table-III among 40 respondents obtaining contraceptives from Health Center, only 7(17.5%) said that contraceptives are not available in Health Center, whereas majority i.e. 33(82.5%) said that contraceptives are always available in Health Center. Majority 27 (67.5%) said that they don't need to pay for obtaining the methods whereas only 13(32.5%) said they need to pay. Among 13 respondents who were claiming health center charges money for contraceptive materials, 5(38.47%) said they gave less than 50 taka, 7(53.85%) said they gave within 51-100 taka and only 1(7.7%) said they gave more than100 taka.

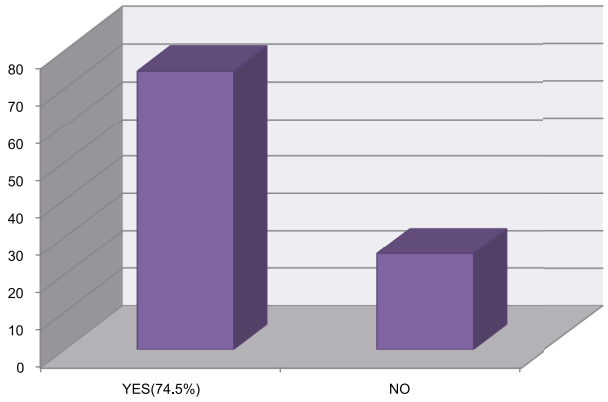


Fig.-7: *ANC received by respondents*

Among 200 respondents, two-third 149(74.5%) said they received ANC during their gestational period and about 51(25.5%) said they didn't receive any ANC during their gestational period.

Discussion:

According to a country report on Bangladesh, a South-South Initiative Permanent Observer at the United Nations, the contraceptive prevalence rate in Bangladesh is 58% of currently married women[1, 2, 6]. Whereas in this present study, the contraceptive prevalence rate was 77.5%.

The most commonly used contraceptive methods according to the some study[1,9] were oral contraceptive pills followed by injectable contraceptives, this result is highly coincide with the findings of the present study.[1,9]

In this study almost all (91.5%) of the women are aware about contraception with the family planning methods which is quite different with the finding of another study conducted in Uganda where only 31 percent of the respondents in Gulu and 18 percent in Luwero reported to have had information about contraception[10, 11]. This may be explained by that Dhamrai is near to capital Dhaka, people are informed enough about their health and family planning.

In this study, the main constraint for not using contraception is that husband did not like it which accounted 18(40%) and other reasons (42%) were like non availability, expensive, and religious factors. In other survey it was found that the constrain of not using the contraceptive methods owing to the side effects related to contraceptives. [4,5,15] Similarly, another studies found that irregular menstrual bleeding and other side effects due to the contraceptive methods led to a discontinuity in its use.[12,13,14]

As far as delivery of family planning devices is concerned, most of the respondents received their contraceptive methods from drug stores. This result is nearly similar with a study conducted in Uganda, most of the people in

some districts of Uganda, obtain the devices from places other than the government health centres.[11] The most obvious reason being drug stores were within their reach and they can easily have access to what they want.

It is revealed that among 200 women, only 34.2% of the women had received ANC, more than or equals to the minimum advised visits by WHO that is 4 times. This result is nearly coincide with a study where 26% of the women in Bangladesh receive ANC.[1]

Conclusion :

The growth curve of the country is on the rise, which is an issue of concern. In the light of the information we have received, the idea about family planning is still not very prevalent among the rural people of the villages. This can only be achieved if existing barriers to access are identified and addressed. Then the reasons which have been posed to not use family planning methods can be understood and be dealt with. By increasing awareness about the different contraceptive methods through health promotion and health education, people can make an informed choice. A large proportion of respondents obtain contraceptive methods from drug store instead of obtaining it from health workers or health centers for free, the government should thus raise more awareness about this matter and ensure its availability. By closer monitoring, planning and policy making can ensure pregnant women to receive at least three ANC which will reduce the MMR, further Increasing maternal knowledge on pregnancy and a healthy delivery will help ensure a decrease in the MMR and IMR.

References:

1. World Population 2012. Department of Economic and Social Affairs, United Nations.
2. Bangladesh Population growth rate. [Available from: www.indexmundi.com]
3. Bangladesh Bureau of Statistics, Household Income and Expenditure Survey (HIES) 2011.
4. Hannan A. Bangladesh Demographic and Health Survey - 2011. National Institute of Population Research and Training (NIPORT), Mitra and Associates and ICF International, 2013.
5. BDHS (Bangladesh Demographic and Health Survey) -2011. National Institute of Population Research and Training (NIPORT), Mitra and Associates, and ICF International,2013.[Availablefom:http://www.niport.gov.bd&http://www.measuredhs.com]
6. Kabir H. Association of programmatic factors with low contraceptive prevalence rates in a rural area of Bangladesh. [Available from: www.reproductive-health-journal.com]
7. Ullah MS, Chakraborty N. Factors affecting the use of contraception in Bangladesh: a multivariate analysis. Asia Pac Popul J. 1993;8(3):19–30.
8. Islam R M, Thorvaldsen G. Family planning knowledge and current use of contraception among the Mru indigenous women in Bangladesh. Open Access Journal of Contraception 2012:3 9–16.
9. Jones J, Mosher W and Daniels K. Current Contraceptive Use in the United States, 2006–2010, and Changes in Patterns of Use Since 1995. National Health and Statistics report.
10. Mills S, Bos E, and Suzuki E. Unmet need for contraception. The consistency and validity of reproductive attitudes: evidence from Morocco, J Biosoc Sci. 1998 (4):439–55; and T.K. Roy et al., Canwomen's childbearing and contraceptive intentions predict contraceptive demand? Family Planning Perspectives 2003, 29 (1): 25–31.
11. Kisaakye P. Determinants of unmet needs of contraception and space limit births among various groups of currently married women in Uganda. 1st Annual International Interdisciplinary Conference, AIIC 2013, 24-26 April, Azores, Portugal.
12. Hardon A. Women's Views and Experiences of Hormonal Contraceptives: What We Know and What We Need to Find Out. Beyond acceptability. 68.
13. Islam MA. Factors affecting current use of contraceptives: a multinomial logistic regression analysis. *J Stat Stud*. 2000; 20: 9–13.
14. Khan MA. Factors associated with oral contraceptive discontinuation in rural Bangladesh. *Health Policy Plan*. 2003;18(1):101–108.
15. Klitsch M. Half of Bangladeshi women who discontinue pill use attribute their decision to side effects. Int Fam Plan Perspect. 2002;28(1):49–50.

Green Life Medical College Journal Vol. 2, No. 1, January 2017

REVIEW ARTICLE

Neurological Manifestations of Inflammatory Rheumatic Disorders: A Review

ISLAM MR[1], RAHMAN T[2], ARA R[3]

Abstract

Patients with rheumatic conditions may present with neurological manifestations, creating challenges in making an appropriate diagnosis & management. A better understanding of the clinical characteristics of various central nervous system features of rheumatologic diseases is pivotal for diagnosis & management. Early assessment is often helpful in averting the development of serious complications, which in some conditions can be prevented by the prompt diagnosis and treatment. We reviewed the spectrum of neurological disease in patients with a rheumatological diagnosis. The wide variety of associated neurological complications is discussed in the context of specific rheumatic conditions, varying from spinal cord involvement in rheumatoid arthritis to neuropsychiatric involvement in systemic lupus erythematosus and neurological sequelae in vasculitic disorders & other rheumatic disorders.

Key words: *Neurological manifestations, Rheumatic disorders*

Journal of Green Life Med. Col. 2017; 2(1): 17-25

Introduction:

Rheumatic diseases are a collection of chronic inflammatory conditions stemming from autoimmune attack on connective tissue. The systemic nature of these diseases can result in secondary Neurologic symptoms, either as a direct result of immunologic attack or by indirect degradation of supporting structures. Neurotoxic effects of treatment can also cause complex neurologic symptoms.[1] Although certain neurological complications such as entrapment neuropathies are common and well recognized.[2] Other associations are less well appreciated. The most prominent features of neurological involvement in rheumatic diseases include cerebral ischemia, polyneuropathy and psychiatric symptoms. Neurological associations in rheumatic diseases should be distinguished from important differential diagnoses, including multiple sclerosis, infection and malignancy. Little information is available on the prevalence of neurological disease in patients with a rheumatological diagnosis. In one study, 100 consecutive patients attending a neurology service were screened.[3] Eleven percent had a rheumatic or autoimmune disorder directly related to their neurological diagnosis. The most common conditions were Sjo¨gren's syndrome (3%) and the presence of lupus anticoagulant (3%). The spectrum of conditions included stroke, dementia, migraine and hemiparetic somatization. Assessment of serum autoantibodies can be helpful, as certain diagnoses are associated with recognized autoantibody profiles.[4, 5]

1. Dr. Md. Rashedul Islam, Registrar, Department of Neurology, BIRDEM General Hospital.
2. Dr. Tanbin Rahman, Registrar, Department of Medicine, Green life Medical College Hospital
3. Dr. Rowsan Ara, Associate Professor, Department of Medicine, Green life Medical College Hospital.

Address of correspondence: Dr. Md Rashedul Islam, Registrar, Department of Neurology (Room No-1423, 13Th Floor), BIRDEM General Hospital, Shahbag, Dhaka-1000, Phone: 01819216209, Email: rashed2k2001@yahoo.com

Methods:

Medline search was done up to December 2015 using the keywords "Neurological manifestations", "Rheumatic disorders" to identify previously published literatures containing information regarding neurological presentations of different inflammatory rheumatic disorders. Articles were checked manually for relevance. Only review articles and original articles in English were considered. Finally, 113 articles were analyzed for this review.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic, progressive, systemic inflammatory disorder where joints are the primary target. A wide spectrum of neurological conditions

occur in RA, including peripheral neuropathy, myelopathy, vasculitis causing neuropathy and stroke, myositis and denervation atrophy.[6,7] Cord compression is one of the most important life-threatening neurological sequelae. It can result not only from atlantoaxial subluxation at the cervical spine, but also from rheumatoid nodules[8,9] and epidural lipomatosis.[10] More rarely, nerve palsies, including hypoglossal nerve paresis,[11] optic neuritis and pachymeningitis are also described.[12]

Cerebral manifestations of RA are infrequent complications of the disease but include rheumatoid nodulosis and vasculitis. Nodules typically develop in the dura, meninges, or choroid plexus but have been reported in the brain parenchyma. Rheumatic nodules are often asymptomatic, so the true incidence and pattern of these lesions remains unknown. Cerebral vasculitis in RA is rare and develops in the meninges, choroid plexus, and parenchyma. Patients with established, seropositive, active RA and other extra-articular manifestations of the disease appear to be at higher risk of developing CNS complications.[13, 14] Abrupt stroke-like episodes with fluctuating hemiparesis have been described without scalp EEG evidence of seizures.[15] Other presentations include cranial neuropathies and seizures.[16] Associated headaches range from mild to very severe. Cerebrospinal fluid analysis typically shows a mononuclear cell predominant pleocytosis (usually mild, < 100 cells/iL), normal to mildly elevated protein, and low to normal glucose.[16,17,18] Typical MRI findings include impressive pachymeningeal enhancement and thickening along with leptomeningeal fluid attenuated inversion recovery (FLAIR) hyperintensities and contrast enhancement.[15,16] The EEG can range from normal to mild nonspecific slowing.[15]

Vasculitis of the CNS with RA is rare and typically affects small caliber arteries.[18] The vasculitis can affect the meninges, gray matter, or white matter, and can occur either isolated to the CNS or as part of systemic involvement.[18,19] The clinical presentation is remarkably varied, ranging from acute to subacute onset of confusion, paresis, seizure, ataxia, cranial neuropathies, or visual dysfunction.[18-21] Laboratory abnormalities include elevated inflammatory markers, such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).[20,21] Cerebrospinal fluid analysis does not reliably show pleocytosis and cannot be used to exclude vasculitis.[18,21] Brain MRI typically shows T2 hyperintensities in the subcortical and periventricular white matter.[19,20,22]

Compared with inflammatory diseases of the brain, cervical spine instability with RA is a much more common complication. The etiology of the tropism within the spinal column remains unclear, though a possible explanation is that the occiput–C1 and C1–C2 joints are exclusively synovial without intervertebral disks like other vertebral segments.[23] The synovial inflammation leads to bony erosion, ligamentous destruction, and pannus formation, which all contribute to structural instability of the spinal column and consequent compression of the spinal cord or vertebral arteries.[24]

Radiological involvement of the cervical spine can be present in up to 86% of all RA patients.[25,26] Instability can be associated with neck pain and/or it can be associated with compression of adjacent structures, particularly the brainstem, the spinal cord, or spinal nerve roots. Damage to the lateral masses of C1 leads to bone loss and vertical translocation of C1 through the foramen magnum, which is termed as basilar invagination. This can cause brainstem compression. The commonest instability is atlantoaxial (AA) subluxation, typically forward subluxation of C1 on C2.[27] This can cause C2 root pain and/or a myelopathy. More than fifty percent of cervical spine deformity occurs at C1/2. [28.29] The remaining 50% occurs in the subaxial cervical spine and this can cause a radiculopathy or myelopathy. Subaxial instability at multiple levels produces a stepladder deformity. Instability is most commonly found in patients who have had RA for ten years or longer; many patients are asymptomatic over an extended period of time. [30,31]

Common clinical symptoms include neck pain (present in ~ 65% of patients with RA) and C2 radiculopathy, which can result in occipital headache or ear pain when involving the auricular branch.[32] More concerning symptoms are those of upper cervical myelopathy, such as difficulty walking, numbness, hyperreflexia, or spasticity. When there is compression of vertebral arteries, signs or symptoms of vertebrobasilar insufficiency can also be present. Many more patients have radiographic evidence of cervical spine disease than have clinical symptoms of myelopathy. The technique used to evaluate the spine affects the estimated prevalence of disease as well. In a series of 40 consecutive patients with RA who underwent both X-ray and MRI, cervical spine disease was noted in 40% of X-rays, but in 70% of cervical spine MRIs.[32] Despite the high prevalence of disease on MRI, myelopathic symptoms were present in only 22% of patients.[32]

Neuropathy in RA is prevalent, but often subclinical. In a recently reported cross-sectional study of 108 patients with RA, 57% had electrodiagnostic evidence of neuropathy,

while only approximately 25% had signs or symptoms of neuropathy on clinical examination.[33] Electrophysiologically, the neuropathy is primarily axonal (85%), though demyelinating features can be present.[33] The neuropathy can encompass pure sensory (most common) or sensorimotor modalities.[34] When sural nerve biopsies are performed, the pathology is remarkably variable, ranging from normal to perineural thickening to perivascular lymphocytic cell infiltrate to demyelination to vasculitis (rare).[33] Entrapment of nerves such as of the median nerve at the carpal tunnel or of the posterior interosseous nerve at the arcade of Frohse is a common etiology of neuropathy.[34,35] Vasculitis in RA is an infrequent cause of neuropathy, though multiple mononeuropathies in a patient with RA is highly suggestive of vasculitis.[33]

RA patients with overt vascular involvement have early-onset cardiovascular disease and are twice as likely to experience myocardial infarction as compared with healthy age-matched controls within a 10-year period.[36] Vascular disease may account for one-third of all mortality in RA patients.[37]

The moderate stroke risk in RA has a late evolution in the course of the disease compared with other cardiovascular complications that are early onset in these patients. RA does not cause large-artery cerebrovascular disease, but systemic inflammation is well established as an accelerant of atherosclerosis in the RA population and probably plays a role in the increased risk of stroke over time; procoagulable factors may be upregulated in established RA, and it is possible that this late-stage increase in hypercoagulability may account for the slow evolution of increased stroke risk in RA patients.[38] In addition, posterior embolic stroke may result from direct occlusion of the vertebrobasilar circulation secondary to rheumatic degradation of the transverse ligament and subsequent atlantoaxial subluxation. Vertebral artery compression due to cervical subluxation occurs in 5% of RA patients; the insidious destruction of the atlantoaxial joint over time also contributes to the late onset of RA stroke risk.[39]

Systemic Lupus Erythematosus

SLE is associated with the highest incidence of neurologic sequelae among all rheumatic diseases. Between 25% and 70% of SLE patients will experience neurologic or psychological symptoms, with 12% of these sequelae established before a diagnosis of SLE and another 12% presenting with neuropsychiatric symptoms at the time of diagnosis.[40] Up to 50% of SLE patients will experience CNS complications. A significant percentage of SLE patients develop distinct neuropsychiatric symptoms during the course of the disease; 17% to 30% will be diagnosed with neuropsychiatric SLE (NPSLE).[41, 42]

Psychiatric symptoms are the commonest form of neurological involvement, [43, 44, 45] but rarer associations such as peripheral nerve conduction abnormalities[46] and transverse myelitis requiring urgent immunosuppressive therapy, also form part of small but significant group.[47] Epileptic seizures are an important feature of CNS lupus. In a study of 519 patients with SLE, 11.6% had epileptic seizures. Seizures occurred at the onset of SLE symptoms in 31.6% and after disease onset in 68.3%.[48] MRI remains the principal brain imaging method in SLE. In one study, 116 MRI scans performed on 85 patients with SLE were reviewed: [49] 60% showed high-signal lesions on T2-weighted images, which most frequently occurred in the frontal and parietal subcortical white matter. Functional brain imaging, including magnetic resonance spectroscopy and diffusion/perfusion weighted imaging, has been used to assess disease activity in SLE.[50] A study using PET in SLE showed hypometabolism in at least one brain region in all patients with severe or mild CNS symptoms (100%), compared with patients without cerebral symptoms (40%).[51]

Vascular complications are observed in 10% to 40% of patients with SLE and can cause multiorgan pathology. The immune complexes observed in SLE are known to activate endothelial cells and elicit a proinflammatory and proliferative response, contributing to the pathologic development of vasculitis.[52] Concurrent antiphospholipid syndrome in SLE patients may play a role in vascular complications as a result of the antiphospholipid syndrome prothrombotic effect, but this is difficult to discern from small vessel vasculopathy.52 SLE patients have an increased risk of stroke, but increasing evidence suggests that CNS complications and myelopathies in lupus are rarely caused by vasculitis. Vasculopathy plays a larger role than simple vessel occlusion in the pathogenesis of peripheral neuropathies in SLE.[53]

Cerebrovascular disease is one of the neuropsychiatric manifestations of SLE. While all neuropsychiatric syndromes in SLE patients have a strong negative impact on morbidity, only cerebrovascular disease and myelopathy are associated with increased risk of mortality[54] while the risk of stroke in SLE shows age-dependent increases along with the general population, the youngest SLE patients have the highest stroke risk in comparison with their healthy conterparts.[55]

Although headache is the most commonly associated neurologic symptom in SLE, it is likely no more prevalent in SLE than in the general population.[56] Neither tension type nor migraine headache is more prevalent in SLE.[57] Headache also does not appear to be a consistent marker of generalized systemic lupus activity.[57]

Similar to headache, depression is commonly reported with SLE, but is also prevalent in the general population. Estimates of prevalence of depression in adults with SLE has ranged from 2 to 60%.[58]

Estimates of seizure prevalence in SLE range from 7 to 40%.[59] Seizures primarily associated with SLE can be generalized or focal in origin and occur either at the onset of SLE or during the course of disease.[60,61] Factors predicting seizures with SLE include a history of stroke and presence of lupus anticoagulant, elevated serum antiphospholipid antibodies, and positive serum anti-Sm antibody.[62] The majority of seizures are single episodes (~88%) and do not result in epilepsy.[60] Higher baseline disease activity is associated with recurrent seizures.[59-62] Although antineuronal antibodies have been associated with seizures, whether to test for them and which ones to test for in a patient with only seizures are unclear. Magnetic resonance imaging often shows cortical atrophy and small periventricular and cortical/subcortical lesions consistent with small vessel disease.[60-62]

Inflammatory myelopathy, sometimes in the form of a catastrophic, longitudinally extensive transverse myelitis, may occur in patients with rheumatologic disease, particularly SLE and primary Sjo¨gren syndrome. As noted by Wingerchuk and Weinshenker [63] Given the similar at-risk population of women of childbearing age, relapsing and remitting demyelinating lesions in the brain may represent concurrent multiple sclerosis or SLE-related demyelinating disease.[64] Lupus-related myelopathy, on the other hand, has been recently proposed to be distinct, with two clinical subsets.[65] The largest cohort described consists of 22 patients presenting with acute myelitis.[66] Half of these patients presented with lower motor neuron findings including flaccidity and hyporeflexia, and rapidly deteriorated to clinical nadir within 6 hours. These lower motor neuron signs persisted over time and did not appear to be transient spinal shock. This pattern of myelitis was associated with a clinical prodrome of fever, active systemic inflammatory disease from SLE, and a cerebrospinal fluid (CSF) profile consisting of a neutrophilic predominant pleocytosis, elevated protein, and low glucose.[66] The other half of the cohort presented with classic signs of white matter disruption myelitis, including spasticity and hyperreflexia. Magnetic resonance imaging of the spinal cord in both patterns of myelitis frequently showed longitudinally extensive lesions spanning more than three vertebral segments. Patients with white matter patterns of myelitis had an associated history of optic neuritis, and approximately 80% satisfied criteria for a neuromyelitis optica (NMO) spectrum disorder.[66]

Estimates of peripheral neuropathy in SLE vary widely ranging from 1% to 13%.[67, 68] among patients with neuropathy, the most common subtypes are polyneuropathy (55%), peripheral mononeuropathy (11%), and cranial neuropathy (12%).[68]

The overlap between SLE and the antiphospholipid syndrome is an important consideration. In some patients with SLE who also have antiphospholipid antibodies, their neurological disease may be attributable to the prothrombotic risk conferred by the presence of these antibodies. The development of strokes and migraine in patients with antiphospholipid antibodies may warrant treatment with anticoagulants, including aspirin, heparin or warfarin, with doses adjusted until the disease activity is stabilized.[69]

Vasculitis

Vasculopathy is common in systemic autoimmune disease and contributes to many of the neurologic presentations in rheumatic disease. Direct autoimmune mediated destruction and chronic systemic inflammation contribute to vascular degradation. Homogenous noninflammatory fibrointimal hyperplasia results in progressive vascular occlusion, causing collateral vessel formation and digital vessel infarction, while polyarteritic lesions result in intravascular occlusion and thrombosis and carry a worse prognosis than noninflammatory lesions.[70, 71]

Takayasu's arteritis

Takayasu's arteritis is a large-vessel vasculitis of the aorta and its branches. CNS symptoms such as light-headedness, dizziness and visual disturbances are described.[72] The absence of peripheral pulses, claudication symptoms, hypertension, age >40 years, raised ESR and imaging of the affected vessels are all criteria in making the diagnosis. Stroke due to vertebral/carotid ischemia or embolism had been described.[72]

Temporal arteritis

Temporal arteritis is a large-vessel vasculitis preferentially affecting the superficial temporal artery and other branches of the external carotid artery. Moderate to severe headache, particularly in the temporal region, was described by most patients.[73] Diplopia, blurred vision or amaurosis fugax

may precede the development of sudden unilateral blindness. Overall, up to 40% of patients experienced visual loss[74] and the most common cause was ischemic optic neuropathy.[75] Diagnosis is aided by high ESR and/or CRP, as well as a temporal artery biopsy that shows a mononuclear cell infiltrate with intimal hyperplasia and occasional giant cells in active disease.[76]

Granulomatosis with Polyangitis (Wegener's granulomatosis)

Granulomatosis with polyangitis is a vasculitis associated primarily with pulmonary and renal involvement. In a large series of 324 patients, 33.6% had neurological involvement. The commonest features included peripheral neuropathies, cranial neuropathy, ophthalmoplegia, cerebrovascular events and seizures.[77]

Polyarteritis nodosa (PAN)

PAN is a form of systemic necrotizing vasculitis that usually presents with some kind of neurological disturbance. The neurological conditions found include mononeuritis multiplex and symmetrical polyneuropathy.[78] Distal sensorimotor polyneuropathy and late CNS involvement leading to encephalopathy were also reported.[79]

Churg-Strauss angiitis

Churg-Strauss angiitis is a necrotizing vasculitis with prominent eosinophilic tissue infiltrates and granulomas affecting medium- and small-sized vessels. A mononeuropathy or polyneuropathy form part of the diagnostic criteria.[80]

Behcets syndrome

Behcets syndrome is a syndrome of recurrent, painful oral and genital lesions associated with uveitis and other forms of systemic inflammation. Neurological involvement is classified into: (i) inflammation of CNS tissue or (ii) vasculitis with a stroke-like presentation and sinus venous thrombosis.[81] The latter form of involvement is less frequent.[82] The wide variety of neurological findings that occur are headaches, ocular and other cranial nerve palsies, seizures, cerebrovascular insufficiency, brainstem syndrome leading to cerebellar ataxia and pseudobulbar palsy.[83] Spinal cord disease, hemisphere lesions and meningoencephalitis also occur.[84]

Sjo¨gren Syndrome

Estimates of neurologic involvement in Sjo¨gren syndrome range from 0%to 70%, but the prevalence was closer to 20%. Peripheral nervous system (PNS) dysfunction in Sjo¨gren syndrome includes symmetric sensorimotor polyneuropathy and cranial neuropathy as common findings. PNS involvement in primary Sjo¨gren syndrome (PSS) has an estimated prevalence of 20%. CNS involvement in primary Sjo¨gren syndrome is less frequent. Approximately 1% to 8% patients showed CNS dysfunction, including mild cognitive deficits, headache, and spinal cord involvement. The majority (63%) of Sjo¨gren syndrome patients with CNS involvement will also have PNS abnormalities. Central deficits precede the diagnosis of primary Sjo¨gren syndrome in many cases. Patients who have primary Sjo¨gren syndrome with neurologic involvement are generally older than those without neurologic symptoms.[84,85,86]

Focal encephalic involvement is the main CNS manifestation in PSS.[87,88] These focal disorders can include motor and sensory loss with hemiparesis, aphasia, dysarthria, seizures, movement disorders, and cerebellar syndrome.[89,90] Spinal cord disorders can include acute or chronic progressive myelopathies, lower motor neuron disease, or neurogenic bladder[88,89,90] Subacute transverse myelitis with high signal on T2 weighted images and abnormal cerebrospinal fluid (CSF) study (increased protein level and cell count) is a rare but well-described complication in PSS patients.[91,92] Optic neuropathies have been also described in PSS.[93] CNS involvement can be diffuse, presenting encephalopathy, cognitive disfunction, dementia, psychiatric abnormalities, and aseptic meningoencephalitis.[94, 95, 96] Cognitive disturbances of variable severity have been described in PSS patients without mood disorders.[97]

Peripheral neuropathy was the most common neurological complication of PSS. It was reported between 20 and 50% of patients when subclinical neuropathy was revealed by a systematic electrophysiological study [98] PNS disease included axonal polyneuropathies (distal axonal sensory and sensorimotor), neuronopathies, mononeuropathies, cranial nerves involvement (mainly trigeminal neuropathy), and autonomic system involvement. Axonal polyneuropathies were the most common manifestations of PNS involvement found in 50% of PNS cases. [99,100]A mixed sensorimotor polyneuropathy, involving large diameter fibers, most commonly axonal, may be present in PSS. The motor neuron involvement (amyotrophic lateral sclerosis syndrome and anterior horn syndrome) is a rare neurological manifestation in PSS [101,102] and may be associated with CNS involvement.[103] Another manifestation is the acute motor axonal neuropathy (AMAN), a variant seen in nearly 5% of Guillain-Barré syndrome. More than 60% of AMAN patients have antibodies against ganglioside M1 (GM1).[103,104] Often

multiple and recurrent cranial nerves neuropathy may be present in PSS. The most common is trigeminal neuropathy, followed by facial and oculomotor nerves involvement.[105, 106]

Scleroderma

Scleroderma is a multisystem disease characterized by skin thickening and vascular disorders. Rare associations include intracerebral inflammation and the 'en coup de sabre'[107,108] variant of scleroderma. Brachial plexopathy[109] and progressive brain atrophy[110] attributed to underlying scleroderma were described.

Sarcoidosis

In the CNS sarcoid, granulomas most often involve the meninges. The commonest findings are cranial nerve involvement, CNS parenchymal disease and demyelination. More rarely, features such as aseptic meningitis and peripheral neuropathy were found. CNS granulomas, symptomatic or asymptomatic, can be seen on T2-weighted MRI or contrast enhanced CT.[111]

Ankylosing spondylitis

Ankylosing spondylitis is an inflammatory arthropathy that affects predominantly the axial skeleton. It often begins in the sacroiliac joints and ascends to involve the remaining spine. The main neurological complications in ankylosing spondylitis occur due to axial disease with spinal cord impingement at multiple levels. Surgical procedures in patients with atlanto-occipital disease, atlantoaxial subluxation and spinal stenosis have been performed for pain and neurological deficit.[112,113]

Conclusion:

Neurological involvement in rheumatic disease is associated with high morbidity and in some cases can be devastating. Early assessment and diagnosis of recognized complications are essential for management of such patients. Having a thorough understanding of the major rheumatic disorders is important when evaluating patients presenting with neurologic manifestations. This is best accomplished by joint effort of neurologists and rheumatologists working as a team.

References:

1. Jonathan M. Goldstein. Neurologic Complications of Rheumatic Disease. CONTINUUM: Lifelong Learning in Neurology 2014; 20(3): 657-669
2. Muhammed N, Campbell P, Smith IS. Peripheral nerve entrapment syndromes: diagnosis and management. Br J Hosp Med 1995; 53:141–6.
3. Olsen ML, O'Connor S, Arnett FC, Rosenbaum D, Grotta JC, Warner NB. Autoantibodies and rheumatic disorders in a neurology inpatient population: a prospective study. Am J Med 1991; 90:479–88.
4. Sellar RJ. Imaging blood vessels of the head and neck. J Neurol Neurosurg Psychiatry 1995; 59:225–37.
5. Verro P, Levine SR, Tietjen GE. Cerebrovascular ischemic events with high positive anticardiolipin antibodies. Stroke 1998; 29:2245–53.
6. Kim RC, Collins GH. The neuropathology of rheumatoid disease. Hum Pathol 1981; 12:5–15.
7. Cupps TR, Fauci AS. The vasculitic syndromes. Adv Intern Med 1982; 27:315–44.
8. Tsegaye M, Bassi S, Ashpole RD. Extradural spinal cord compression by rheumatoid nodule. Br J Neurosurg 2003; 17:255–7.
9. Fairburn B. Spinal cord compression by a rheumatoid nodule. J Neurol Neurosurg Psychiatry 1975; 38:1056–8.
10. Arroyo IL, Barron KS, Brewer EJ Jr. Spinal cord compression by epidural lipomatosis in juvenile rheumatoid arthritis. Arthr Rheum 1988; 31:447–5.
11. Castro S, Verstraete K, Mielants H, Vanderstraeten G, de Reuck J, Veys EM. Cervical spine involvement in RA: a clinical, neurological and radiological evaluation. Clin Exp Rheumatol 1994; 12:369–74.
12. Agildere AM, Tutar NU, Yucel E, Coskum M, Benli S, Aydin P. Pachymeningitis and optic neuritis in rheumatoid arthritis: MRI findings. Br J Radiol 1999; 72:404–7.
13. Caballol Pons N, Montala N, Valverde J, et al. Isolated cerebral vasculitis associated with rheumatoid arthritis. Joint Bone Spine 2010;77(4):361Y363.
14. Guadalupe Loya-de la Cerda D, Avile´ s-Soli´s JC, Delgado-Montemayor MJ, et al. Isolated rheumatoid arthritis-associated cerebral vasculitis: a diagnostic challenge. Joint Bone Spine 2013;80(1):88Y90.
15. Bourgeois P, Rivest J, Bocti C. Rheumatoid meningitis presenting with stroke-like episodes. Neurology 2014;82(17):1564–1565
16. Yuh WT, Drew JM, Rizzo M, Ryals TJ, Sato Y, Bell WE. Evaluation of pachymeningitis bycontrast-enhanced MR imaging in a patient with rheumatoid disease. AJNR Am J Neuroradiol 1990;11(6):1247–1248
17. Bathon JM, Moreland LW, DiBartolomeo AG. Inflammatory central nervous system involvement in rheumatoid arthritis. Semin Arthritis Rheum 1989;18(4):258–266
18. Kato T, Hoshi K, Sekijima Y, et al. Rheumatoid meningitis: an autopsy report and review of the literature. Clin Rheumatol 2003;22(6):475–480
19. Ramos M, Mandybur TI. Cerebral vasculitis in rheumatoid arthritis. Arch Neurol 1975;32(4):271–275
20. Ando Y, Kai S, Uyama E, et al. Involvement of the central nervous system in rheumatoid arthritis: its clinical manifestations and analysis bymagnetic resonance imaging. InternMed 1995;34(3):188–191
21. Guadalupe Loya-de la Cerda D, Avilés-Solís JC, Delgado-Montemayor MJ, Camara-Lemarroy CR, Galarza-Delgado DÁ.

Isolated rheumatoid arthritis-associated cerebral vasculitis: a diagnostic challenge. Joint Bone Spine 2013;80(1):88–90

22. Singleton JD, West SG, Reddy VV, Rak KM. Cerebral vasculitis complicating rheumatoid arthritis. South Med J 1995;88(4):470–474
23. da Côrte FC, Neves N. Cervical spine instability in rheumatoid arthritis. Eur J Orthop Surg Traumatol 2014;24(Suppl 1):83–91
24. Mukerji N, Todd NV. Cervicalmyelopathy in rheumatoid arthritis. Neurol Res Int 2011;2011:153628
25. P. M. Pellicci, C. S. Ranawat, P. Tsairis, and W. J. Bryan, "A prospective study of the progression of rheumatoid arthritis of the cervical spine," Journal of Bone and Joint Surgery—Series A, vol. 63, no. 3, pp. 342–350, 1981.
26. P. H. Smith, J. Sharp, and J. H. Kellgren, "Natural history of rheumatoid cervical subluxations,"Annals of the Rheumatic Diseases, vol. 31, no. 3, pp. 222–223, 1972.
27. A. W. B. Heywood, I. D. Learmonth, and M. Thomas, "Cervical spine instability in rheumatoid arthritis," Journal of Bone and Joint Surgery—Series B, vol. 70, no. 5, pp. 702–707, 1988.
28. Y. Morizono, T. Sakou, and H. Kawaida, "Upper cervical involvement in rheumatoid arthritis," Spine, vol. 12, no. 8, pp. 721–725, 1987.
29. H. V. Nguyen, S. C. Ludwig, J. Silber et al., "Rheumatoid arthritis of the cervical spine," Spine Journal, vol. 4, no. 3, pp. 329–334, 2004.
30. S. Santavirta, D. Hopfner Hallikainen, P. Paukku, J. Sandelin, and Y. T. Konttinen, "Atlantoaxial facet joint arthritis in the rheumatoid cervical spine. A panoramic zonography study," Journal of Rheumatology, vol. 15, no. 2, pp. 217–223, 1988.
31. M. Schmitt-Sody, C. Kirchhoff, S. Buhmann et al., "Timing of cervical spine stabilisation and outcome in patients with rheumatoid arthritis," International Orthopaedics, vol. 32, no. 4, pp. 511–516, 2008.
32. Younes M, Belghali S, Kriâa S, et al. Compared imaging of the rheumatoid cervical spine: prevalence study and associated factors. Joint Bone Spine 2009;76(4):361–368
33. Agarwal V, Singh RWiclaf, et al. A clinical, electrophysiological, and pathological study of neuropathy in rheumatoid arthritis. Clin Rheumatol 2008;27(7):841–844
34. Biswas M, Chatterjee A, Ghosh SK, Dasgupta S, Ghosh K, Ganguly PK. Prevalence, types, clinical associations, and determinants of peripheral neuropathy in rheumatoid patients. Ann Indian Acad Neurol 2011;14(3):194–197
35. Ostrowski RA, Takagishi T, Robinson J. Rheumatoid arthritis, spondyloarthropathies, and relapsing polychondritis. Handb Clin Neurol 2014;119: 449–461
36. Hayton M, Federation of European Societies for Surgery of the Hand. Vascular and neurological considerations in rheumatoid arthritis. Presented at: 11th Congress of the Federation of European Societies for Surgery of the Hand; June 2006;Glasgow, Scotland.
37. Solte´ sz P, Kerekes G, De´ r H, et al. Comparative assessment of vascular function in autoimmune rheumatic diseases: considerations of prevention and treatment. Autoimmun Rev 2011;10(7):416-425.
38. Holmqvist M, Gra¨nsmark E, Mantel A, et al. Occurrence and relative risk of stroke in incident and prevalent contemporary rheumatoid arthritis. Ann Rheum Dis 2013;72(4):541-546.
39. Kuroki T, Ueno Y, Takeda I, et al. Recurrent embolic strokes associated with vertical atlantoaxial subluxation in a patient with rheumatoid arthritis: a case report and review of literature. J Stroke Cerebrovasc Dis 2013;22(8):676-681.
40. Cikes N. Central nervous system involvement in systemic connective tissue diseases. Clin Neurol Neurosurg 2006;108(3):311-317.
41. Hanly JG, Urowitz MB, Su L, et al. Prospective analysis of neuropsychiatric events in an international disease inception cohort of patients with systemic lupus erythematosus. Ann Rheum Dis 2010;69(3):529-535.
42. Wang M, Gladman DD, Iban˜ ez D, UrowitzMB. Long-term outcome of early neuropsychiatric events due to active disease in systemic lupus erythematosus. Arthritis Care Res (Hoboken) 2012;64(6):833-837.
43. The ACR nomenclature and case definitions for neuropsychiatric lupus syndromes. Arthritis Rheum 1999; 42:599–608.
44. Feinglass EJ, Arnett FC, Dorsch CA, Zizic TM, Stevens MB. Neuropsychiatric manifestations of systemic lupus erythematosus: diagnosis, clinical spectrum and relationship to other features of the disease. Medicine 1976; 55:323–39.
45. Loukkola J, Laine M, Ainiala H, Peltola J, Metsanoja R, Auvinen A, Hietaharju A. Cognitive impairment in SLE and neuropsychiatric SLE: a population-based neuropsychological study. J Clin Exp Neuropsychol 2003; 25:145–51.
46. McNicholl JM, Glynn D, Mongey AB, Hutchinson M, Bresnihan B. A prospective study of neurophysiologic, neurologic and immunologic abnormalities in SLE. J Rheumatol 1994; 21:1061–6.
47. Kovacs B, Lafferty TL, Brent LH, DeHoratius RJ. Transverse myelopathy in SLE: an analysis of 14 cases and review of the literature. Ann Rheum Dis 2000; 59:120–4.
48. Appenzeller S, Cendes F, Costellat LT. Epileptic seizures in systemic lupus erythematosus. Neurology 2004; 63:1808–12.
49. Ramos-Casals M, Brito-Zero´ n P. Emerging biological therapies in primary Sjogren's syndrome. Rheumatology (Oxford) 2007;46(9):1389Y1396.
50. St Clair EW,LevesqueMC, Prak ET, et al. Rituximab therapy for primary Sjo¨ gren's syndrome: an open-label clinical trial and mechanistic analysis. Arthritis Rheum 2013;65(4):1097Y1106.
51. Aringer M, Burkhardt H, Burmester GR, et al. Current state of evidence on 'off-label' therapeutic options for systemic lupus erythematosus, including biological immunosuppressive agents, in Germany, Austria and SwitzerlandVa consensus report. Lupus 2012;21(4):386-401.
52. Ferreira S, D'Cruz DP, Hughes GR. Multiple sclerosis, neuropsychiatric lupus and antiphospholipid syndrome: where do we stand? Rheumatology (Oxford) 2005;44(4): 434-442.

53. Monov S, Monova D. Classification criteria for neuropsychiatric systemic lupus erythematosus: do they need a discussion? Hippokratia 2008;12(2):103-107.

54. Wang M, Gladman DD, Iban˜ ez D, UrowitzMB. Long-term outcome of early neuropsychiatric events due to active disease in systemic lupus erythematosus. Arthritis Care Res (Hoboken) 2012;64(6):833-837.

55. Krishnan E. Stroke subtypes among young patients with systemic lupus erythematosus. Am J Med 2005;118(12):1415.

56. Mok CC, To CH, Mak A. Neuropsychiatric damage in Southern Chinese patients with systemic lupus erythematosus. Medicine (Baltimore) 2006;85(4):221–228

57. Mitsikostas DD, Sfikakis PP, Goadsby PJ. A meta-analysis for headache in systemic lupus erythematosus: the evidence and the myth. Brain 2004;127(Pt 5):1200–1209

58. Palagini L, Mosca M, Tani C, Gemignani A, Mauri M, Bombardieri S. Depression and systemic lupus erythematosus: a systematic review. Lupus 2013;22(5):409–416

59. Olazarán J, López-Longo J, Cruz I, Bittini A, Carreño L. Cognitive dysfunction in systemic lupus erythematosus: prevalence and correlates. Eur Neurol 2009;62(1):49–55

60. Conti F, Alessandri C, Perricone C, et al. Neurocognitive dysfunction in systemic lupus erythematosus: associationwith antiphospholipid antibodies, disease activity and chronic damage. PLoS ONE 2012;7(3):e33824

61. Kowal C, Degiorgio LA, Lee JY, et al. Human lupus autoantibodies against NMDAreceptors mediate cognitive impairment. Proc Natl Acad Sci U S A 2006;103(52):19854–19859

62. Devinsky O, Schein A, Najjar S. Epilepsy associated with systemic autoimmune disorders. Epilepsy Curr 2013;13(2): 62–68

63. Wingerchuk DM, Weinshenker BG. Acute disseminated encephalomyelitis, transverse myelitis, and neuromyelitis optica. Continuum (Minneap Minn) 2013;19(4 Multiple Sclerosis): 944-967.

64. Oomatia A, Fang H, Petri M, Birnbaum J. Peripheral neuropathies in systemic lupus erythematosus: clinical features, disease associations, and immunologic characteristics evaluated over a twenty-five-year study period. Arthritis Rheumatol 2014;66(4):1000–1009

65. Birnbaum J, Petri M, Thompson R, Izbudak I, Kerr D. Distinct subtypes of myelitis in systemic lupus erythematosus. Arthritis Rheum 2009;60(11):3378–3387

66. Birnbaum J, Petri M, Thompson R, Izbudak I, Kerr D. Distinct subtypes of myelitis in systemic lupus erythematosus. Arthritis Rheum 2009;60(11):3378–3387

67. Unterman A, Nolte JES, Boaz M, Abady M, Shoenfeld Y, Zandman-Goddard G. Neuropsychiatric syndromes in systemic lupus erythematosus: a meta-analysis. Semin Arthritis Rheum 2011;41(1):1–11

68. Florica B, Aghdassi E, Su J, Gladman DD, Urowitz MB, Fortin PR. Peripheral neuropathy in patients with systemic lupus erythematosus. Semin Arthritis Rheum 2011;41(2):203–211

69. Sanna G, Bertolaccini ML, Mathieu A. Central nervous system lupus: a clinical approach to therapy. Lupus 2003; 12:935–42.

70. Hayton M, Federation of European Societies for Surgery of the Hand. Vascular and neurological considerations in rheumatoid arthritis. Presented at: 11th Congress of the Federation of European Societies for Surgery of the Hand; June 2006;Glasgow, Scotland.

71. Caballol Pons N, Montala N, Valverde J, et al. Isolated cerebral vasculitis associated with rheumatoid arthritis. Joint Bone Spine 2010;77(4):361Y363.

72. Arend WP, Michel BA, Bloch DA, Hunder GG, Calabrese LH, Edworthy SM, Fauci AS, Leavitt RY, Lie JT, Lightfoot RW Jr, et al. The American College of Rheumatology 1990 criteria for the classification of Takayasu's arteritis. Arthritis Rheum 1990; 33:1129–34.

73. Hunder GG, Bloch DA, Michel BA, Stvens MB, Arend WP, Calabrese LH, Edworthy SM, Fauci AS, Leavitt RJ, Lie JT, et al. The American College of Rheumatology 1990 criteria for the classification of giant cell arteritis. Arthritis Rheum 1990; 33:1122–8.

74. Huston KA, Hunder GG, Lie JT, Kennedy RH, Elveback LR. Temporal arteritis, a 25-year epidemiologic, clinical and pathological study. Ann Intern Med 1978; 88:162–7.

75. Reich KA, Giansiracusa DF, Strongwater SL. Neurologic manifestations of giant cell arteritis. Am J Med 1990; 89:67–72.

76. Schmidt WA, Blockmans D. Use of ultrasonography and positron emission tomography in the diagnosis and assessment of large-vessel vasculitis. Curr Opin Rheumatol 2005; 17:9–15.

77. Nishino H, Rubino FA, DeRemee RA, Swanson JW, Parisi JE. Neurological involvement in Wegener's granulomatosis: an analysis of 324 consecutive patients at the Mayo Clinic. Ann Neurol 1993; 33:4–9.

78. Lightfoot RW, Michel BA, Bloch DA, Hunder GG, Zvaifler NJ, McShane DJ, Arend WP, Calabrese LH, Leavitt RY, Lie JT et al. The American College of Rheumatology 1990 criteria for the classification of polyarteritis nodosa. Arthritis Rheum 1990; 33:1088–93.

79. Walker GL. Neurological features of polyarteritis nodosa. Clin Exp Neurol 1978; 15:237–47.

80. Masi AT, Hunder GG, Lie JT, Michel BA, Bloch DA, Arend WP, Calabrese LH, Edworthy SM, Fauci AS, Leavitt RY, et al. The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). Arthritis Rheum 1990; 33:1094–100.

81. International Study Group for Behcet's disease. Criteria for diagnosis of Behc¸et's disease. Lancet 1990; 335:1078–80.

82. Siva A, Altintas A, Saip S. Behc¸et's syndrome and the nervous system. Curr Opin Neurol 2004; 17:347–57.

83. Kidd D, Steuer A, Denman AM, Rudge P. Neurological complications in Behc¸et's syndrome. Brain 1999; 122:2183–94.

84. Massara A, Bonazza S, Castellino G, et al. Central nervous system involvement in Sjo¨gren's syndrome: unusual, but not

unremarkableVclinical, serological characteristics and outcomes in a large cohort of Italian patients. Rheumatology (Oxford) 2010;49(8):1540Y1549.

85. Cikes N. Central nervous system involvement in systemic connective tissue diseases. Clin Neurol Neurosurg 2006;108(3):311Y317.

86. Tobo´ n GJ, Pers JO, Devauchelle-Pensec V, Youinou P. Neurological disorders in primary Sjo¨ gren's syndrome. Autoimmune Dis 2012;2012: 645967.

87. E. L. Alexander, T. T. Provost, M. B. Stevens, and G. E. Alexander, "Neurologic complications of primary Sjögren's syndrome," Medicine, vol. 61, no. 4, pp. 247–257, 1982.

88. S. Delalande, J. De Seze, A. L. Fauchais et al., "Neurologic manifestations in primary Sjögren syndrome: a study of 82 patients," Medicine, vol. 83, no. 5, pp. 280–291, 2004.

89. K. Morgen, H. F. McFarland, and S. R. Pillemer, "Central nervous system disease in primary Sjögren's syndrome: the role of magnetic resonance imaging," Seminars in Arthritis and Rheumatism, vol. 34, no. 3, pp. 623–630, 2004.

90. F. C. Soliotis, C. P. Mavragani, and H. M. Moutsopoulos, "Central nervous system involvement in Sjögren's syndrome," Annals of the Rheumatic Diseases, vol. 63, no. 6, pp. 616–620, 2004.

88. S. Delalande, J. De Seze, A. L. Fauchais et al., "Neurologic manifestations in primary Sjögren syndrome: a study of 82 patients," Medicine, vol. 83, no. 5, pp. 280–291, 2004.

89. Y. T. Konttinen, E. Kinnunen, and M. Von Bonsdorff, "Acute transverse myelopathy successfully treated with plasmapheresis and prednisone in a patient with primary Sjögren's syndrome," Arthritis and Rheumatism, vol. 30, no. 3, pp. 339–344, 1987.

90. C. S. Williams, E. Butler, and G. C. Román, "Treatment of myelopathy in Sjögren's syndrome with a combination of prednisone and cyclophosphamide," Archives of Neurology, vol. 58, no. 5, pp. 815–819, 2001.

91. Y. Kaneko, A. Suwa, A. Nakajima et al., "A case of primary Sjögren's syndrome accompanied by transverse myelitis," Ryumachi, vol. 38, no. 4, pp. 600–604, 1998.

92. R. K. Lyu, S. T. Chen, L. M. Tang, and T. C. Chen, "Acute transverse myelopathy and cutaneous vasculopathy in primary Sjögren's syndrome," European Neurology, vol. 35, no. 6, pp. 359–362, 1995.

93. P. Rapoport, H. Merle, D. Smadja, M. Gerard, and E. Alliot, "Bilateral optic neuropathy disclosing primary Gougerot-Sjögren syndrome," Journal Français d'Ophtalmologie, vol. 20, no. 10, pp. 767–770, 1997.

94. E. L. Alexander and G. E. Alexander, "Aseptic meningoencephalitis in primary Sjögren's syndrome,"Neurology, vol. 33, no. 5, pp. 593–598, 1983.

95. R. P. Gerraty, P. A. Mckelvie, and E. Byrhe, "Aspetic meningoencephalitis in primary Sjögren's syndrome: response to plasmapheresis and absence of CNS vasculitis at autopsy," Acta Neurologica Scandinavica, vol. 88, no. 4, pp. 309–311, 1993.

96. F. Moutaouakil, B. El Moutawakkil, H. El Otmani, I. Gam, M. A. Rafai, and I. Slassi, "Aseptic meningoencephalitis in primary Gougerot-Sjögren's syndrome," Revue Neurologique, vol. 161, no. 12, pp. 1225–1227, 2005.

97. C. Lafitte, Z. Amoura, P. Cacoub et al., "Neurological complications of primary Sjögren's syndrome,"Journal of Neurology, vol. 248, no. 7, pp. 577–584, 2001.

98. A. P. Andonopoulos, G. Lagos, A. A. Drosos, and H. M. Moutsopoulos, "The spectrum of neurological involvement in Sjögren's syndrome," British Journal of Rheumatology, vol. 29, no. 1, pp. 21–23, 1990.

99. S. I. Mellgren, D. L. Conn, J. C. Stevens, and P. J. Dyck, "Peripheral neuropathy in primary Sjögren's syndrome," Neurology, vol. 39, no. 3, pp. 390–394, 1989.

100. S. Delalande, J. De Seze, A. L. Fauchais et al., "Neurologic manifestations in primary Sjögren syndrome: a study of 82 patients," Medicine, vol. 83, no. 5, pp. 280–291, 2004.

101. H. Attout, F. Rahmeh, and F. Ziegler, "Syndrome de Gougerot-Sjögren simulant une sclérose latérale amyotrophique," Revue de Médecine Interne, vol. 21, no. 8, pp. 708–710, 2000.

102. H. Mochizuki, K. Kamakura, T. Masaki, A. Hirata, R. Nakamura, and K. Motoyoshi, "Motor dominant neuropathy in Sjögren's syndrome: report of two cases," Internal Medicine, vol. 41, no. 2, pp. 142–146, 2002.

103. S. Vucic, M. C. Kiernan, and D. R. Cornblath, "Guillain-Barré syndrome: an update," Journal of Clinical Neuroscience, vol. 16, no. 6, pp. 733–741, 2009.

104. R. A. C. Hughes and D. R. Cornblath, "Guillain-Barré syndrome," The Lancet, vol. 366, no. 9497, pp. 1653–1666, 2005.

105. D. Vincent, P. Loron, A. Awada, and J. C. Gautier, "Recurrent multiple cranial nerve palsies. Goujerot-Sjögren's syndrome," Revue Neurologique, vol. 141, no. 4, pp. 318–321, 1985.

106. P. Bakouche, J. P. Ferroir, and A. Guillard, "Multiple and recurrent paralysis of cranial nerves: primary Gougerot-Sjögren syndrome," Revue Neurologique, vol. 150, no. 10, pp. 728–731, 1994.

107. Stone J, Franks AJ, Guthrie JA, Johnson MH. Scleroderma 'en coup de sabre': pathological evidence of intracerebral inflammation. J Neurol Neurosurg Psychiatry 2001; 70:382–5.

108. Gambichler T, Kreuter A, Hoffman K, Bechara FG, Altmeyer P, Jansen T. Bilateral linear scleroderma 'en coup de sabre' associated with facial atrophy and neurological complications. BMC Dermatol 2001; 1:9.

109. Allanore Y, Zuber M, Kahan A. Brachial plexopathy associated with systemic sclerosis. Clin Rheumatol 2002; 21:401–2.

110. Grosso S, Fioravanti A, Biasi G, Conversano E, Marcolongo R, Morgese G, Balestri P. Linear scleroderma associated with progressive brain atrophy. Brain Dev 2003; 25:57–61.

111. Stern BJ. Neurological complications of sarcoidosis. Curr Opin Neurol 2004; 17:311–16.

112. Fox MW, Onofrio BM, Kilgore JE. Neurological complications of ankylosing spondylitis. J Neurosurg 1993; 78:871–8.

113. Ramos-Remus C, Gomez-Vargas A, Guzman-Guzman JL, Jimenez-Gil F, Gamez-Nava JI, Gonzalez-Lopez L, Farrera-Gamboa H, Maksymowych WP, Suarez-Almazor ME. Frequency of atlantoaxial subluxation and neurologic involvement in patients with ankylosing spondylitis. J Rheumatol 1995; 22:2120–5.

REVIEW ARTICLE

Gene Therapy: A Review Article

YESMIN K[1], KABIR F[2], ALAM S[3], BEGUM S[4], HASSAN MMM[5]

Abstract

Gene therapy can be broadly defined as the transfer of genetic material to cure a disease or at least to improve the clinical status of a patient. Gene therapy is the therapeutic delivery of nucleic acid polymers into patient's cells as a drug to treat disease. Currently, gene therapy studies a broad range of potential therapeutic interventions, including the body's immune reaction to tumors, new blood vessels in the heart to alleviate heart attacks and to stop HIV-replication in patients with AIDS. There is also renewed emphasis on the gene therapy of genetic diseases, such as hemophilia A and B, and cystic fibrosis. Human gene therapy experimentation raises many issues. In this review article, introduction, background of gene therapy,gene therapy strategies, approaches of gene therapy, methods of gene therapy, somatic gene therapy-ex vivo, in vitro and in vivo-gene therapy, germ line gene therapy, vectors in gene therapy, advantages and disadvantages of gene therapy, risks associated with gene therapy and journey of clinical trials in gene therapy have been given.

Key Words: Gene Therapy, methods, Somatic gene therapy, Vectors

Journal of Green Life Med. Col. 2017; 2(1): 26-34

Introduction:

James Watson was quoted as saying "we used to think that our fate was in our stars, but now we know, in large measures, our fate is in our genes". Genes, the functional unit of heredity, are specific sequences bases that encode instructions to make proteins. Although genes get a lot of attentions, it is the proteins that perform most life functions. When genes are altered, encoded proteins are unable to carry out their normal functions, resulting in genetic disorders. Gene therapy (use of genes as medicines) is basically to correct defective genes responsible for genetic disorder by one of the following approaches:[1, 2]

1. Dr. Kazi Yesmin, Associate Professor of Microbiology, Green Life Medical College.
2. Dr. Fahmida Kabir, Associate Professor of Biochemistry, Green Life Medical College.
3. Dr. Sahbub Alam, Assistant Professor of ENT, National Institute of ENT.
4. Dr. Shahanara Begum, Professor of Microbiology, Green Life Medical College.
5. Dr. M.M. Monzur Hassan, Professor of Microbiology, Green Life Medical College.

Address of Correspondence: Dr. Kazi Yesmin, Associate Professor of Microbiology, Green Life Medical College, E-mail: kaziyesmin@gmail.com

- A normal gene could be inserted into a nonspecific location within the genome to replace the Nonfunctional gene (most common)
- An abnormal gene could be swapped for a normal genehomologous recombination
- An abnormal gene could be repaired through selective reverse mutation
- Regulation (degree to which a gene is turned on or off) ofa particular gene could be altered

Gene therapy states and remains an experimental discipline and many researches remain to be performed before the treatment will realize its potential. Majority of the gene therapy trials are being conducted in United States and Europe, with only a modest number in other countries including Australia. Scope of this approach is broad with potential in treatment of diseases caused by single gene recessive disorders (like cystic fibrosis, hemophilia, muscular dystrophy, sickle cell anemia etc), acquired genetic diseases such as cancer and certain viral infections like AIDS.[3,4]

Other gene therapy projects are targeted at conditions such as heart disease, diabetes mellitus, arthritis and Alzheimer's disease, all of which involve genetic susceptibility to illness.[5] Table 1 shows a summary of approved current clinical gene therapy protocols.

Background:

Since the earliest days of plant and animal domestication, about 10,000 years ago, humans have understood that characteristics traits of parents could be transmitted to their offspring. The first to speculate about how this process worked were ancient Greek scholars, and some of their theories remained in favor for several centuries. The scientific study of genetics began in 1850s, when Austrian monk Gregor Mendel, in a series of experiments with green peas, described the pattern of inheritance, observing that traits were inherited as separate units we know as genes. Mendel's work formed the foundation for later scientific achievements that heralded the era of modern genetics. But little was known about the physical nature of genes until 1950s, when American biochemist James Watson and British biophysicist Francis Crick developed their revolutionary model of double stranded DNA helix. Another key breakthrough came in the early 1970s, when researchers discovered a series of enzymes that made it possible to snip apart genes at predetermined site along a molecule of DNA and glue them back together in a reproducible manner. Those genetic advances set the stage for the emergence of genetic engineering, which has produced new drugs and antibodies and enabled scientists tocontemplate gene therapy. A few years after the isolation of genes from DNA, gene therapy was discovered in 1980s.[6]

Strategies of gene therapy[7]

Gene augmentation therapy**,** a DNA is inserted into the genome to replace the missing gene products **(**Fig-1**).**

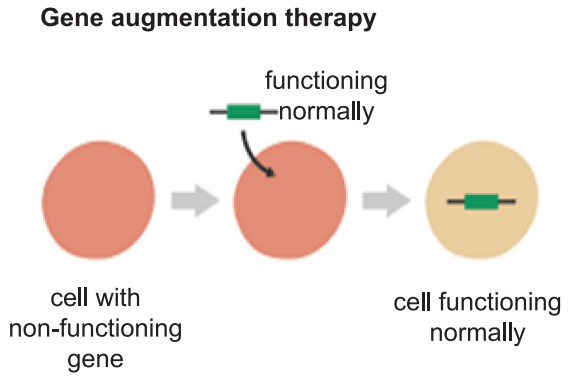


Fig.-1

Gene inhibition therapy**,** the antisense gene inhibits the expression of the dominant gene **(**Fig-2**)**.

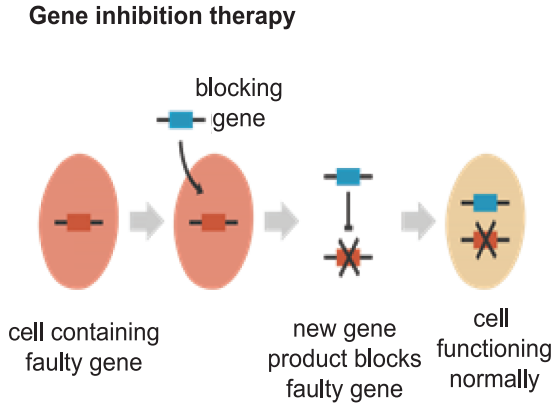


Fig.-2

Approaches of gene therapy[8]

1. Gene modification
   - i) Replacement therapy
   - ii) Corrective Gene therapy
2. Gene transfer
   - i) Physical
   - ii) Chemical
   - iii) Biological
3. Gene transfer in specific cell line
   - i) Somatic gene therapy
   - ii) Germ line gene therapy

4. Eugenic approach (gene insertion) Other forms of genetic engineering include gene targeting and knocking out specific genes via engineered nucleases such as zinc finger nucleases, engineered I-CreI homing endonucleases, or nucleases generated from TAL effectors. This approach is cur-rently being used in several human clinical trials.

In somatic gene therapy involves the insertion of a fully functional and expressible gene into a target somatic cell to correct a genetic disease permanently. The non reproductive cells of an organ are referred to as somatic cells. These include bone marrow cell, blood cells, skin cell & intestinal cell. There are two types of somatic gene therapies - i**)** In vivo gene therapy which involves the genetic material is transferred directly into the body of the patient **(**Fig-3**).**

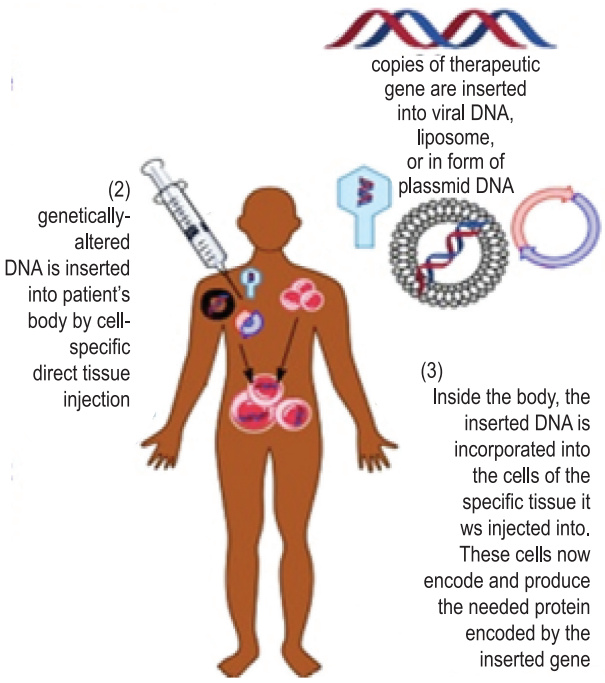


Fig.-3

ii) Ex vivo gene therapy in which the genetic material is first transferred into the cells grown in outside the body (Fig-4).

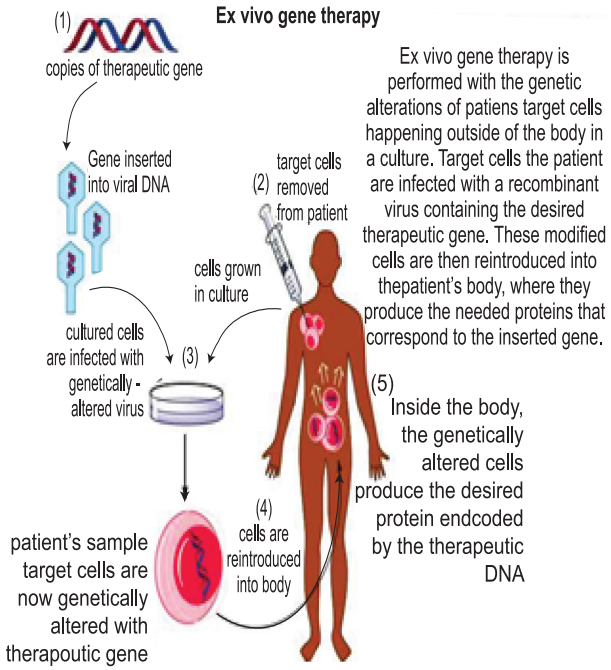


Fig.-4

In this method genetically altered cells are selected, expanded and the cells are then returned back to the patient e.g, hepatocytes, skin fibroblasts, haematopoietic cells.[7]

In germ line gene therapy the reproductive cells of an organ constitutes germ cell line. Gene therapy involving the introduction of DNA into germ cells is passed on to the successive generations. For safety, ethical and technical reasons, germ cell gene therapy is not being attempted at present.

Vectors used in gene therapy

Some of the different types of viruses used as gene therapy vectors (Fig-5):

Retroviruses - A class of viruses that can create double-stranded DNA copies of their RNA genomes. These copies of its genome can be integrated into the chromosomes of host cells. Human immunodeficiency virus (HIV) is a retrovirus.

One of the problems of gene therapy using retroviruses is that the integrase enzyme can insert the genetic material of the virus into any arbitrary position in the genome of the host; it randomly inserts the genetic material into a chromosome. If genetic material happens to be inserted in the middle of one of the original genes of the host cell, this gene will be disrupted (insertional mutagenesis). If the gene happens to be one regulating cell division, uncontrolled cell division (i.e., cancer) can occur. This problem has recently begun to be addressed by utilizing zinc finger nucleases[9] or by including certain sequences such as the beta-globin locus control region to direct the site of integration to specific chromosomal sites.

Adenoviruses - A class of viruses with double-stranded DNA genomes that cause respiratory, intestinal, and eye infections in humans. The virus that causes the common cold is an ade-novirus.

Adeno - associated viruses- A class of small, single-stranded DNA viruses that can insert their genetic material at a specific site on chromosome 19.[10]

Herpes simplex viruses - A class of double-stranded DNA viruses that infect a particular cell type, neurons. Herpes simplex virus type 1 is a common human pathogen that causes cold sores.[11]

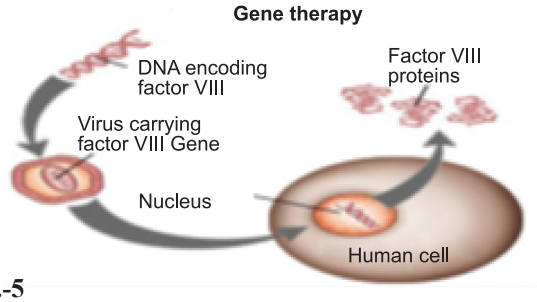


Fig.-5

Non-viral methods present certain advantages over viral methods, with simple large scale production and low host immunogenicity being just two. Previously, low levels of transfection and expression of the gene held non-viral methods at a disadvantage; however, recent advances in vector technology have yielded molecules and techniques with transfection efficiencies similar to those of viruses. Ormasil (organically modifided silica or silicate) used as non-viral method.[12]

Injection of naked DNA

This is the simplest method of non-viral transfection. Clinical trials carried out of intramuscular injection of a naked DNA plasmid have occurred with some success; however, the expression has been very low in comparison to other methods of transfection.

Physical methods to enhance delivery :

Electroporation

Electroporation is a method that uses short pulses of high voltage to carry DNA across the cell membrane. This shock is thought to cause temporary formation of pores in the cell membrane, allowing DNA molecules to pass through. Electroporation is generally efficient and works across a broad range of cell types. However, a high rate of cell death follow-ing electroporation has limited its use, including clinical appli-cations.

Gene Gun

The use of particle bombardment, or the gene gun, is another physical method of DNA transfection. In this technique, DNA is coated with gold particles and loaded into a device which generates a force to achieve penetration of DNA/gold into the cells.eg:- If the DNA is integrated in the wrong place in the genome, for example in a tumor suppressor gene, it could induce a tumor **(**Fig-6**)**.[13]

Fig.-6

Sonoporation

Sonoporation uses ultrasonic frequencies to deliver DNA into cells.

Magnetofection

In this method, DNA is complexed to a magnetic particles, and a magnet is placed underneath the tissue culture dish to bring DNA complexes into contact with a cell monolayer.[14]

Chemical methods to enhance delivery

- **Oligonucleotides -** The use of synthetic oligonucleotides in gene therapy is to inactivate the genes involved in the disease process. There are several methods by which this is achieved. One strategy uses antisense specific to the target gene to disrupt the transcription of the faulty gene.
- **Lipoplexes and polyplexes -** To improve the delivery of the new DNA into the cell, the DNA must be protected from dam-age and (positively charged). Initially, anionic and neutral lipids were used for the construction of lipoplexes for synthetic vectors **(**Fig-7**)**.

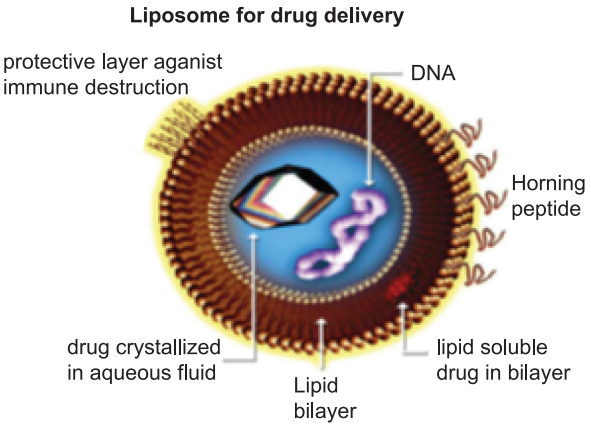


Fig-7

- **Dendrimers -** A dendrimer is a highly branched macromolecule with a spherical shape. When in the presence of genetic material such as DNA or RNA, charge complimentarity leads to a temporary association of the nucleic acid with the cationic dendrimer. On reaching its destination the dendrimer nucleic acid complex is then taken into the cell via endocytosis.
- **Hybrid methods -** Due to every method of gene transfer having shortcomings, there have been some

hybrid methods developed that combine two or more techniques. Virosomes are one example; they combine liposomes with an inactivated HIV or influenza virus. This has been shown to have more efficient gene transfer in respiratory epithelial cells than either viral or liposomal methods alone. [15]

Advantages and disadvantages of gene therapy:

Advantages of gene therapy

- In case of 'silence' a gene. In the case of someone with HIV, which had not yet developed into AIDS, scientists could save them the pain and suffering of the disease by using gene ther-apy to 'silence' the disease before its onset.
- Gene therapy has the potential to eliminate and prevent he-reditary diseases such as cystic fibrosis and is a possible cure for heart disease, AIDS and cancer.
- These sceptics would almost certainly choose gene therapy, especially if it was the last hope for them or one of their loved ones – as is the case for many gene therapy patients.[16,17]

Disadvantages of Gene Therapy

- Short-lived nature of gene therapy.
- Immune response - Genes injected with a virus may trigger an immune response against the virus. Problems with viral vectors (once inside the patient, the viral vector could recover its ability to cause disease).
- Multigene disorders - The genetic material might not get into the right cell, or the right place in the cell's DNA.[18]

Risks associated with gene therapy:

Viruses can infect more than one cell types. The external gene might be inserted into the wrong location in the DNA, causing cancer or other damage. When DNA is injected directly into a tumor, there is a risk some DNA to be introduced into germ cells, producing inheritable changes. The gene might be over-expressed (toxicity).The viral vector could cause inflammation or immune reaction. The virus could be transmitted to other individuals or the environment.[19]

Ethical issues surrounding gene therapy are:

- Who decides which traits are normal and which constitute a disability or disorder?
- Will the high costs of gene therapy make it available only to the wealthy?
- Could the widespread use of gene therapy make society less accepting of people who are different?
- Should people be allowed to use gene therapy to enhance basic human traits such as height, intelligence, or athletic ability?

Journey of clinical trials in gene therapy [1980 – 2016]

There are several early speculations on the method of gene therapy.[20] In 1966, Tatum predicted that viruses could be used to convert genes in theoretical studies in somatic-cell genetics. The first attempt in genetics was done in Pecking ducklings which were injected with DNA extracts from Khaki Campbell ducks and expressed some of the characteristics of the said duck but another trial on albino rat by DNA extracts of pigmented rat did not produce any significant result. In 1970, American doctor Stanfield Rogers tried to treat two sisters, suffering from arginemia (that lacks enzyme arginase, a type of protein) by injecting shope papilloma virus containing an arginase gene but this gene therapy was unsuccessful to raise the above protein levels higher. In 1977, scientists were able to use gene therapy technique to deliver a gene into cells of mammals.

In 1980, Mercola and Cline undertook the first human gene therapy trial to treat â thalassemia patients by transfecting â globin gene into human bone marrow cells.[21] This protocol lacked appropriate ethical review, was widely reviewed as premature on scientific grounds and was eventually stopped. Two important points emerged from this study. One was the fact that highly regulated and coordinated expression of both Ü-like and â-like globin genes would likely be required for successful gene therapy of the hemoglobinopathies. Other was the need to address the safety and ethical concern adequately in clinical trials of gene therapy.

In 1990, American doctor Anderson performed one of the first successful gene therapy study on a 4 year old girl named Ashanti DeSilva, with a rare genetic immune system disorder called severe combined immuno-deficiency (SCID). The lack of production of adenosine deaminase (ADA), had made her immune system weak, so she had become susceptible to many severe diseases. Anderson and his colleagues extracted her WBCs, implanted genes producing ADA into WBCs and then transferred the cells back to her body. The WBCs strengthened the girl's immune system made it possible for her to survive. The effects were only temporary, but successful.[22]

In 1992, Claudio Bordignon of Italy performed the first procedure of gene therapy using hematopoietic stem cells

as vectors to deliver genes intended to correct hereditary disease.[23]

In 1993, a new born baby Andrew Gobea, with SCID, was treated by gene therapy technique using retrovirus vector carrying ADA gene.[24] Blood was removed from his placenta and umbilical cord immediately after birth, containing stem cells. Retrovirus (carrying ADA gene) and stem cells were mixed, after which they entered and inserted the gene into stem cells' chromosome. Stem cells containing the working ADA enzyme were also given weekly. For next few years, WBCs (produced by stem cells), made ADA enzyme using ADA gene. After then further treatment was needed.

In 1999, gene therapy suffered a major setback with the death of 18 year old Jesse Gelsinger who participated in a gene therapy trial for ornithine transcarboxylase deficiency.[18] He died from multiple organ failure 4 days after starting the treatment. His death was believed to have been triggered by a severe immune response to the adenovirus carrier.

In 2002, French researcher Alain Fischer tried to cure children suffering from X- linked SCID (also known as bubble boy) by inserting retrovirus carrying normal gene into children's blood stem cell. This clinical trial was questioned when 2 of them developed a leukemia-like condition.[25] However another major blow came in Jan 2003, when the "FOOD and DRUG ADMINISTRATION" [FDA] placed a temporary halt on all gene therapy trials using retrovirus vector in blood stem cells.

Then in April 2003, FDA eased the ban after regulatory review of the protocol in USA, UK, France, Italy and Germany[26] since the treatment had benefitted a large number of children.

Researchers at Case Western Reserve University and Copernicus Therapeutics have been able to create DNA nanoballs (tiny liposomes 25nm) that can carry therapeutic DNA through pores in nuclear membrane. Moreover gene therapy approach repairs errors in messengerRNA derived from defective genes. This technique has the potential to treat thalassemia, cystic fibrosis, and some forms of cancers.

In 2003, Los Angeles research team inserted genes into brain using liposome coated in a polymer called polyethylene glycol.[27] The transfer of gene into brain is a significant achievement because viral vectors are too big to get across the blood brain barrier. This method has potential for treatment for Parkinson's disease.

Scientists at the National Institute of Health (Bethesda, Maryland) have successfully treated metastatic melanoma in two patients using killer T cells genetically retargeted to attack the cancer cells. This study constitutes one of the first demonstrations that gene therapy can be effective in treating cancer. In another development, gene therapy may be used to Huntington's disease.

Short interfering RNAs (siRNAs) are designed to match the RNA, copied from a faulty gene and to produce abnormal protein product of that gene. This RNA interference or gene silencing may be used in gene therapy to switch off Huntington's disease.[28]

In 2005, scientists were able to repair deafness in guinea pig by using adenovirus vector.[29] Atoh1 gene (which stimulates hair cell's growth) was delivered to cochlea resulting in regrowth of hair cells and so regaining 80% of original hearing threshold. This study may pave the way to human trials of gene therapy in such cases.

In 2006 (March), an international group of scientists announced the successful use of gene therapy to treat two adult patients for a disease affecting myeloid cells.[30] Study published in Nature Medicine, is believed to be the first to show that gene therapy can cure disease of myeloid system. In May 2006, a team of scientists from Italy, reported a breakthrough for gene therapy in which they developed a way to prevent immune system from rejecting a newly delivered gene with the use of micro RNAs, whose natural function could be used to selectively turn off the identity of the therapeutic gene.[31] The researchers were successful in mice experimentation. This work will have important implication for the treatment of hemophilia and other genetic disease by gene therapy. In August 2006, researchers successfully reengineered immune cells, called lymphocyte, to target and attack cancer cells in patients with advanced metastatic melanoma. This is the first time that gene therapy is used to successfully treat cancer in humans. In November 2006, Preston Nix from the University of Pennsylvania School of Medicine reported on VRX496, a gene-based immunotherapy for the treatment of human immunodeficiency virus (HIV) that used a lentiviral vector for delivery of an antisense gene against the HIV envelope. Patients responded to the above therapy showing stable and increased immune response (CD4 T cell count). This was the first evaluation of a lentiviral vector administered in U.S. Food and Drug Administration-approved human clinical trials for any disease.[32] Data from an ongoing clinical trial were presented at CROI (conference on retrovirus and opportunistic infection).

In 2007, a team of British doctors from Moorefield's Eye Hospital and University college of London, announced the world's first gene therapy trial to test a revolutionary gene therapy treatment for a type of inherited retinal disease i.e. Leber's congenital amaurosis, which is caused by mutation in the RPE65 gene. Sub-retinal delivery of recombinant AAV carrying RPE65 yielded positive result, with patient having modest increase in vision, and more importantly, no apparent side-effect.[33]

In 2009 (March), the School of Pharmacy in London tried nanotechnology based gene therapy (which delivers genes wrapped in nanoparticles) to target and destroy hard-to-reach cancer cells.[34] In September 2009, journal Nature reported that researchers at the University of Washington and University of Florida were able to give trichomatic vision to squirrel monkey using gene therapy.[35] This could have a significance on future treatment for colour blindness in humans. In November 2009,the journal Science reported that researchers succeeded at halting a fatal brain disease, adrenoleukodystrophy, using a vector derived from HIV to deliver the gene for the missing enzyme.[36]

In 2010, a paper by Komaromy et al. published in April 2010, deals with gene therapy for a form of achromatopsia (complete colour blindness) in dogs. It is presented as idle model to develop gene therapy directed to cone photoreceptor. Cone function and day vision have been restored for at least 33 months in two young dogs with achromatopsia. However, the therapy was less efficient for older dogs.[37]

In 2007 and 2008, a man was cured of HIV by repeated hematopoietic stem cell transplantation with double-delta-32 mutation which disables the CCR5 receptor. This cure was accepted by the medical community in 2011.[38]

In 2011 Neovasculgen was registered in Russia as the first-in-class gene-therapy drug for treatment of peripheral artery disease, including critical limb ischemia.[39,40]

In July 2012, the European Medicines Agency recommended approval of a gene therapy treatment for the first time in either Europe or the United States.

In December 2012, it was reported that 10 of 13 patients with multiple myeloma were in remission "or very close to it" three months after being injected with a treatment involving genetically engineered T cells to target proteins NY-ESO-1 and LAGE-1.[41]

In 2013 three of five subjects who had acute lymphocytic leukemia (ALL) had been in remission for five months to two years after being treated with genetically modified T cells which was reported in march 2013.[42]

In July researchers reported promising results for six children with two severe hereditary diseases had been treated with a partially deactivated lentivirus to replace a faulty gene.[43]

In October two children born with adenosine deaminase severe combined immunodeficiency disease (ADA-SCID) had been treated with genetically engineered stem cells 18 months previously and that their immune systems were showing signs of full recovery. Another threechildren were making progress.[44]

Also in October researchers reported that they had treated six haemophilia sufferers in early 2011 using an adeno-associated virus. Over two years later all six were producing clotting factor.[45]

In 2014 January researchers reported that six choroideremia patients had been treated with adeno-associated virus with a copy of REP1. Over a six-month to two-year period all had improved their sight.[46, 47]

Clinical trials of gene therapy for sickle cell disease were started in 2014 although one review failed to find any such trials.[48]

In 2015 February LentiGlobin BB305, a gene therapy treatment undergoing clinical trials for treatment of beta thalassemia gained FDA "breakthrough.[12]In October, researchers announced that they had treated a baby girl, Layla Richards, with an experimental treatment using donor T-cells genetically engineered using TALEN to attack cancer cells. Two months after the treatment she was still free of her cancer.[49]

In 2016 April the Committee for Medicinal Products for Human Use of the European Medicines Agency endorsed a gene therapy treatment called Strimvelis and recommended it be approved.[50, 51] This treats children born with ADA-SCID and who have no functioning immune system. This would be the second gene therapy treatment to be approved in Europe. [52]

In October, Chinese scientists reported they had started a trial to genetically modify T-cells from 10 adult patients with lung cancer and reinject the modified T-cells back into their bodies to attack the cancer cells. [53, 54]

Conclusion:

Scientists believe that after 20 years, GT would be the last cure of every genetic disease. Now a draft of the human genome map is complete, research is focusing on the function of each gene and the role of the faulty gene play in disease. *'Gene therapy will ultimately change our lives.'*

Table I

Summery of approved and published current clinical gene therapy protocols[55]

Disorder	Objective	Target cells	Mode of delivery	Countries with protocols
ADA deficiency	ADA replacement	Blood	Retrovirus	Italy, the Netherlands, United States
Alpha-1-antitrypsin deficiency	Alpha-1-antitrypsin replacement	Respiratory epithelium	Liposome	United States
AIDS	Antigen presentation HIV inactivation	Blood, marrow blood, marrow	Retrovirus	United States
Cancer	Immune function enhancement	Blood, marrow, tumor	Retrovirus, Liposome, electroporation, cell-mediated transfer	Austria, China, France, Germany, Italy, the Netherlands, United States
	Tumor ablation	Tumor	Retrovirus, non-complexed DNA, cell-mediated transfer	United States
	Chemoprotection	Blood, marrow	Retrovirus	Canada, France, Sweden, United States
	Stem –cell marking	Blood, marrow, tumor	Retrovirus	United States
Cystic fibrosis	Cystic fibrosis transmembrane regulatory enzyme	Respiratory epithelium	Adenovirus, liposome	United Kingdom, United States
Familiar hypercholesterolemia	Replacement of low-density lipoprotein receptors	liver	Retrovirus	United States
Fanconi's anemia	Complement group C gene delivery	Blood, marrow	Retrovirus	United States
Gaucher's disease	Glucocerebrosidase replacement	Blood, marrow	Retrovirus	United States
Hemophilia B	Factor IX replacement	Skin fibroblasts	Retrovirus	
Rheumatoid arthritis	Cytokine delivery	Synovium	Retrovirus	United States

The future of gene therapy:

Gene therapy has not offered any permanent cure to any human patients, a breakthrough may come anytime.A day may come when almost every disease will have a gene therapy, as one of the treatment modalities.Gene therapy may revolutionize the practice of medicine

References:

1. Miller DA. Human gene therapy comes of age. *Nature* 1992;375:455-460.
2. Verma IM, Weitzman MD. Gene therapy: Twenty-first centurymedicine. *Annu Rev Biochem*2005;74:711-738.
3. Knoell DM, Yiu IM. Human gene therapy for hereditary diseases:a review of trials. *Am J Health SystPharma*1998;55:899-904.
4. GinterEK.Gene therapy of hereditary disease. *Vopr Med Khim*2000;46:265-78.
5. Vandendriessche T. Recent developments in gene therapy. *Verh Kacad Geneeskd Belg* 2004;66:305-15.
6. Friedmann T. A brief history of gene therapy. *Nature Genetics*1992;2:93-98.
7. Satyanarayana U, Chakrapani U. Gene therapy. Biochemistry 4th edition, India: Elsever; 2013: pp 625-633.
8. Urnov F.D., Rebar E.J., Holmes M.C., Zhang H.S., Gregory P.D. Genome editing with engineered zinc finger nu-cleases. 2010; 636-646.
9. Durai S., Mani M., Kandavelou K., Wu J., Porteus M.H., Chandrasegaran S. *Nucleic Acids Res.*(2009);33 ,5978-90.
10. Harwood, Adrian J. Protocols for Gene Analysis.
11. Friedmann T., Roblin R. Science1972;175 (25), 949.

12. Alvarez-Erviti L, Seow Y, Yin HF *et al*. *Delivery of siR-NA to the mouse brain by* systemic injection of targeted exo-somes; 2011.

13. Wrobel I. and Collins D. Biochim. Biophys. Acta.1995;1235, 296-304.

14. Woods N.B, Bottero V, Schmidt M, von Kalle C., Verma I.M. Gene therapy: therapeutic gene causing lymphoma. Nature. 2006;440,7088.

15. Wang Hongjie, Dmitry M. Shayakhmetov, Tobias Leege, MichaelHarkey, Qiliang Li, ThaliaPapayannopoulou, George Stamatoyannopolous, and André Lieber. Journal of Virology.2005;79(17), 10999-101.

16. Gao X. and Huang L. (1996) Biochemistry35, 1027.

17. Horn P.A, Morris J.C, Neff T, KiemH.P.Mol. Ther.2004;10(3), 417-31.

18. Jenkins R.G, Meng Q.H, Hodges R.J, Lee L.K, Bottoms S.E.W, Laurent G.J, Willis D, AyaziShamlou P, McAnulty R.J. and Hart S.L. Farhood H, Serbina N. and Huang L. Biochim. Biophys. Acta. 1995;1235, 289-295.

19. Akhtar N, Akram M, Asif HM, Usmanghani K, Shah SMA, Rao SA. Gene therapy: A review article. Journal of Medicinal plants research, 2011; 5 (10); pp1812-1817.

20. F riedmann T, Roblin R. Gene therapy for human genetic disease *Science* 1972;175:949.

21. MercolaKE,Cline MJ. The potential of inserting new genetic information. *N Engl J Med* 1980;292:1297-1300.

22. Blaese RM. Development of gene therapy for immunodeficiency:adenosinedeaminase deficiency. Pediatr Res1993;33(suppl):S49-S55.

23. Abott A. Gene therapy.Italians first to use stem cells. Nature.1992;356:465-99.

24. Kohn DB, et al. Engraftment of gene-modified umbilical cord blood cells in neonates with adenosine deaminase deficiency. Nat Med1995;1:1017-1023.

25. Hacein-Bey-Abina S, et al. LMO2-associated clonal T cell proliferation in two patients after gene therapy for XSCID-X1. Science 2003;302:415-419

26. Cavazzana-CalvoM,Thrasher A, Mavilio F. The future of gene therapy. Nature 2004;427:779-81.

27. Pardridge WM. Tyrosine Hydroxylase Replacement in Experimental Parkinson's disease with Transvascular gene therapy. Neuro Rx.2005;2:129-138.

28. Koutsilieric E, Rethwilm A, Scheller C. The Therapeutic potential of siRNA in gene therapy of neurodegenerative disorder. J Neural Transm Suppl.2007;72:43-49.

29. Hankenson FC, Wathen AB, Eaton KA, Miyazawa T, Swiderski DL, Raphael Y. Guinea pig Adenovirus infection Does not Inhibit Adenoviral vector in a model of Hearing loss. Comp Med.2010;60:130-135.

30. Ott MG, Schmidt M, Schwarzwaelder K, et al. Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EV11, PRDM16 or SETBP1. Nat Med2006;12:401-409.

31. B rown BD, Venneri MA, Zingale A, SergiSergi L, Naldini L. Endogenous microRNA regulation suppresses transgene expression in hematopoietic lineages and enables stable gene transfer. Nat Med2006;12:585-91.

32. Levine BL, Humeau LM, Boyer J, et al. Gene transfer in humans using a conditionally replicating lentiviral vector. ProcNatleAcadSci USA2006;103:17372-7.

33. Maguire AM, Simonelli F, Pierce EA, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med2008;358:2240-8.

34. Chisholm EJ, Vassaux G, Duque PM,Chevre R, Lambert O, Pitard B, et al. Cancer specific transgene expression mediated by systemic injection of nanoparticle. Cancer Res.2009;69:2655.

35. Nature.com (http://www.nature.com/news/2009/09016/full/news.2009.921.html).

36. GeneTherapy Halts Brain Disease in Two Boys-Science now (http://sciencenow.sciencemag.org/cgi/content/full/2009/1105/1).

37. Komaromy A, Alexander J, Rowalon J, Garcia M, Chiodo V,Kaya A, et al. Gene therapy rescues cone function in congenitalachromatopsia. Human Molecular Genetics 2010;19:2518-2593.

38. Rosenberg, Tina (29 May 2011) The Man Who Had HIV and Now Does Not, *New York*.

39. AdisInsightVascular endothelial growth factor gene therapy - HSCI2016; Page accessed 5 June

40. Kutter, Susanne Die 1-Million-Euro-Spritze. wiwo.de.2015

41. Coghlan, AndySouped-up immune cells force leukaemia into remission. New Scientist. Retrieved 15 April 2013

42. Coghlan, Andy.Gene therapy cures leukaemia in eight days. The New Scientist. Retrieved 15 April 2013

43. Biffi A, Montini E, Lorioli L, Cesani M, Fumagalli F, Plati T *et al*. "Lentiviral Hematopoietic Stem Cell Gene Therapy Benefits Metachromatic Leukodystrophy". Science.2013;341 (6148): 1233158.

44. Geddes, Linda.'Bubble kid' success puts gene therapy back on track. The New Scientist. Retrieved 2 November 2013

45. Fischer A, Hacein-Bey-Abina S, Cavazzana-Calvo M. "20 years of gene therapy for SCID". Nature Immunology.2010;11 (6): 457–460.

46. MacLaren R. E, Groppe M, Barnard A R, Cottriall C. L, Tolmachova, T, Seymour, L *et al*. "Retinal gene therapy in patients with choroideremia: Initial findings from a phase 1/2 clinical trial". The Lancet.2014;383(9923): 1129–37.

47. Beali, Abigail.Gene therapy restores sight in people with eye disease The New Scientist. Retrieved 25 January 2014

48. Olowoyeye, A; Okwundu, C. I."Gene therapy for sickle cell disease". Cochrane Database of Systematic Reviews. October 2015. 11 (10): CD007652.

49. "Ten things you might have missed Monday from the world of business". Boston Globe. 3 February 2015. Retrieved 13 February 2015.

50. Kutter, Susanne Die 1-Million-Euro-Spritze. wiwo.de.2015

51. "Summary of opinion1 (initial authorisation) Strimvelis". European Medicines .2016.

52. Hirscheler Ben. "Europe gives green light to first gene therapy for children". Reuters. Retrieved 13 April 2016.

53. Coghlan, Andy "Gene Therapy Approved" The New Scientist.2016; 3068. 8–9.

54. Cyranoski, David. "Chinese scientists to pioneer first human CRISPR trial". Nature 9 2016;535 (7613): 476–477.

55. Dude ID, Cournoyer D. Gene therapy: gene therapy: here to stay, Can Med Asso J 1995; 152:1605-1613.

CASE REPORT

Acute Ischemic Stroke in a Pediatric Patient

MALEK A[1], KHAN S[2], DATTA A[3], AKHTER K[4], AHMED S[5], JAHAN SA[6], RAHMAN T[7], HOSSAIN T[8], ARA R[9]

Abstract

Acute ischemic stroke in a pediatric patient is a complex disease with a variety of etiologies that differ from adults. Though rare, they are a real phenomenon with potentially devastating consequences. Some institutions are using anti-thrombotic drug therapy with unclear benefits. Available literature, which is limited to case reports and retrospective reviews of databases, clouds this topic with both positive and negative outcomes. Emergency department management should focus on stabilization and resuscitation with immediate involvement of a pediatric neurologist and intensivist. The decision to use anti-thrombotic drug therapy, including anti-platelet drugs and thrombolytics, should be in consultation with the specialists involved until randomized controlled trials determine their safety and efficacy in the pediatric population.

Key words: Stroke, Ischemic stroke, Pediatric patient.

Journal of Green Life Med. Col. 2017; 2(1): 35-38

Introduction:

Acute ischemic stroke (AIS) in a pediatric patient is a rare medical emergency with an incidence of only 2–3 per 100,000.[1] Cognitive and behavioral sequelae frequently arise with social implications and effects on daily living.[2] The etiologies of stroke in a child are more varied than in adults and are not always due to acute clot formation or hemorrhage. Literature on this topic, including the use of anti-thrombotic therapy for children presenting with AIS, is sparse. Studies in the adult population show anti-thrombotic drugs, including aspirin, are effective when administered as per recommended guidelines.[3] The role of thrombolytics is controversial with conflicting evidence.[4,5,6] This case report discribes a 5-year-old girl who diagnosed as a case of acute ischemic stroke. After extensive work up, the etiology of her stroke remained unclear. At one-month follow up, the patient had only minimal right arm weak less. Background information on AIS in the pediatric population is presented, including emergency department (ED) management and the role of anti-thrombotic drug therapy.

Case presentation:

Baby Jamila, 5-year-old girl admitted on 10th December 2016 in Green Life Medical College Hospital with sudden

Fig: *Right sided hemiplegia with facial drop*

1. Dr. Abdul Malek, Assistant Professor, Department of Pediatrics, Green Life Medical College, Dhaka
2. Dr. Sheela Khan, Assistant Professor, Department of Community Medicine, Green Life Medical College, Dhaka
3. Dr. Anindita Datta, Assistant Professor, Department of Radiology & Imaging, BSMMU.
4. Dr. Khaleda Akhter, Assistant Professor, Department of Pediatrics, City Medical College, Gazipur
5. Dr. Sharmin Ahmed, Assistant Professor, Department of Medicine, Green Life Medical College, Dhaka
6. Dr. Shahina Akhter Jahan, Registrar, Department of Medicine, Green Life Medical College, Dhaka
7. Dr. Tanbin Rahman, Registrar, Department of Medicine, Green Life Medical College.
8. Dr. Tanjina Hossain, Assistant Professor, Department of Medicine, Green Life Medical College, Dhaka
9. Dr. Rowsan Ara, Associate Professor, Department of Medicine, Green Life Medical College, Dhaka

Address of Correspondence: Dr. Abdul Malek, Assistant Professor, Department of Pediatrics, Green Life Medical College, Dhaka, Mobile: 01711983198, E-mail: malekabdul95@yahoo.com

onset of headache, right-sided weakness and dysarthria. Her vital signs were within normal limits. Right arm and leg strength were 2/5 with a noticeable facial droop.

Cardiac examination showed a regular rate and rhythm with no murmurs. Chest was clear and abdomen was soft. No petechiae were noted on her skin.

Fingerstick blood glucose was normal. A MRI of Brain showed acute infract at left capsule-ganglionic region, corona-radiata and medial part of the left temporal lobe. The patient was given aspirin, empiric intravenous antibiotics and antiviral medications. A thorough work-up ensued. The lumbar puncture, ECG, CBC, chemistry panel, liver enzymes, cardiac enzymes and urine screen were normal, as were the SLE panel, Protein C and S, anti-phospholipid antibodies, and coagulation profile, thyroid function, blood and CSF cultures. An MRA of the brain showed moderate narrowing of left middle cerebral artery with no evidence of intracranial aneurysm or flow limiting occlusion.Carotid Doppler and echocardiogram were normal. The patient remained stable for the first two days and regained some motor strength. The patient expended significant time and effort with a physical therapist. By the time of discharge, she had regained the ability to walk. At her one-month follow-up, she had mild residual right arm weakness with mild deficits in her gait but no deficit in speech.

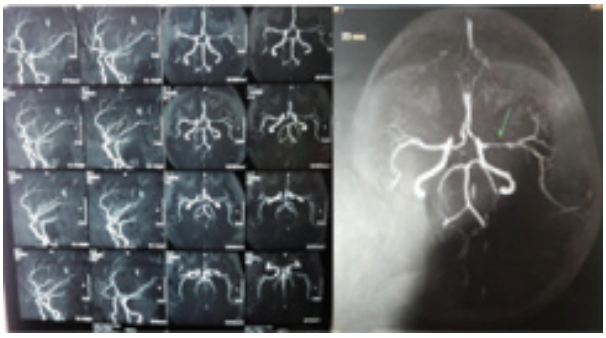


Fig: *MRI showing acute infract at left capsule-gangloinic region, corona-radiata & medial part of the left temporal lobe.*

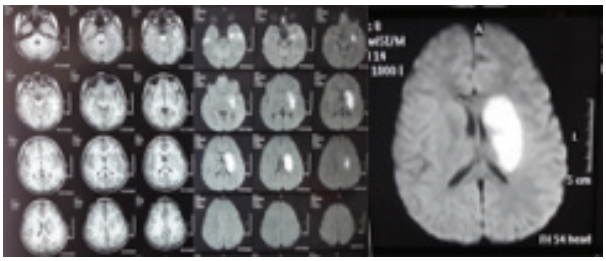


Fig: *MRA showing Moderate narrowing of Mi segment of left middle cerebral artery with no evidence of intracranial aneurysm or occlusion.*

Discussion:

AIS in a pediatric patient is defined as a stroke occurring between the ages of one month and 18 years. This relatively rare condition varies geographically with occurrence in the United States at 2–3 per 100,000 patients. In contrast, the overall incidence rate for total stroke (first-ever and recurrent of all ages) was 269 per 100,000 population.[7] Eighty percent of adult strokes are due to ischemia. While ischemic strokes in adults are usually thrombotic or embolic in nature, they may also be caused by hypoperfusion states. Cryptogenic strokes comprise 30 to 40% of all adult ischemic strokes and approximately 50% in children.[2,8]

Acute ischemic strokes in children most commonly occur between the ages of 1–5 years and least commonly in the extremes (< 1 year and > 15 years). Focal neurological signs occur in three-quarters of patients, with hemiplegia the most common. Despite the fact that infection accounts for 26% of cases, fever is present only 11% of the time. The risk factors for stroke in children are congenital heart disease, infection, prothrombotic disorders, trauma, acquired and congenital vascular disease, sickle cell disease, metabolic disorders and mitochondrial disease. Vascular disease alone accounts for one-third of cases, while metabolic disorders and prothrombotic disorders comprise 18% and 13% of cases respectively. Trauma is responsible for an additional 11% of cases. At least one risk factor is present in 90% of patients with almost 25% of patients having more than one.[9]

There appears to be demographic variation with a predilection for both gender and ethnicity. A seasonal variation may exist, as the disease most commonly occurs during the summer and least commonly during the winter.[9] African-Americans have a significantly higher risk of suffering from both ischemic stroke and hemorrhagic strokes.[10] Males carry a significantly higher risk of suffering from all stroke subtypes compared to females.[10]

Emergency department management of a child suffering from an ischemic stroke should focus on stabilization and transfer to an appropriate facility for specialty care. Stabilization begins with securing the airway, providing supplemental oxygen, establishing IV access and monitoring vitals signs and mental status. An emergent CT of the head in the ED should not be delayed. An ECG, CXR and lab work should be sent, including a complete blood count, blood and CSF cultures, chemistry, liver enzymes, cardiac markers, coagulation factors, urine analysis and urinary drug screen. Empiric intravenous antibiotics and antivirals, maintenance fluid with an isotonic, non-dextrose containing fluid must be initiated in the ED.

Anti-platelet drugs are widely used in adults after literature has shown they reduce the rate of strokes.[2] Although randomized controlled trials with children have not been conducted, anti-platelet drugs are used in some centers to reduce the recurrence rate of stroke.[2] Adverse effects, such as severe bleeding or the precipitation of Reye's syndrome, are rare.[2] The use of anti-thrombotic drugs remains controversial in the pediatric literature and is not the standard of care.[3] However, anti-thrombotic drugs are being given to pediatric patients at some institutions despite a paucity of supporting literature. The benefits are unclear, and it seems that their use is based on adult studies, case reports and expert opinion.[11] One study showed 1.6% of pediatric AIS patients admitted between 2000 and 2003 received thrombolytic therapy.[1] The children receiving thrombolytics had significantly higher medical costs, were less likely to be discharged home and had higher overall mortality rates.[1] Shortcomings of these findings were noted, including the small sample size and unknown severity of any of the patients at the time of presentation. Conversely, several case reports have been published showing potential benefits using thrombolytic therapy in children, including success stories across a wide age range and administration of thrombolytics well outside of the standard three-hour window used in adults.[11,12]

There are numerous etiologies for acute ischemic stroke in the pediatric population. Arterial dissection is one important cause. While most dissections occur in the internal carotid artery, children may dissect intracranially. MRI and contrast MRA show the anatomy without the risks of traditional angiography or radiation. Duplex ultrasonagraphy is useful, but CT angiogram still remains the gold standard for further investigation for suspected arterial dissection.[13] Secondly, cerebral venous sinus thrombosis may cause strokes in children. This commonly develops from extension of infections, including acute otitis media, mastoiditis, pharyngitis, sinusitis, or meningitis. If infection is suspected, appropriate intravenous antimicrobials including a third-generation cephalosporin, vancomycin and acyclovir should be initiated. Additionally, infectious vasculitis may occur from chronic infections such as tuberculous meningitis or rickettsial infection. Vasculitis may also be seen with common viral illnesses such as varicella and coxsackie. Lastly, cerebral vasculitis should be considered in children with either ischemic or hemorrhagic stroke, as well as strokes associated with fever or rash.[13] While erythrocyte sedimentation rate may be used as a screening tool, its interpretation should be used with caution since it does not exclude all vascular etiologies for ischemic stroke. There is no literature to support the empiric use of steroids; however, they may be given to treat cerebral edema or a vasculitic cause of stroke.

The rate of recurrence for childhood stroke may be as high as 30% and is dependent upon the etiology of the stroke.[2] Children with a hypercoagulable disorder or a vascular diagnosis have a higher likelihood of recurrent stroke. Sickle cell disease patients have a 40% chance of repeated stroke, while children with arterial dissection have a recurrence rate of 12%.[13]

Children who suffer AIS generally recover better than adults, but the effects may still be long lasting and detrimental.[14] While children who suffer a stroke have good educational and mobility outcomes, they have poorer outcomes when it comes to communication, socialization and activities of daily living.[14] Social and economic consequences, including decreased ability to work and lifelong disability, may arise. This is even more profound in children having a recurrence. The financial impact of these consequences has yet to be analyzed

Conclusion:

AIS in the pediatric patient is a rare but potentially devastating disease. The lack of research in this area is apparent. There is only anecdotal evidence for the use of anti-thrombotic drugs in the management of children with ischemic strokes, unlike the evidence for treating adult ischemic strokes. Due to the high rate of recurrence anti-platelet drugs should be considered and initiated in the ED.

References:

1. Janjua N, et al. Thrombolysis for ischemic stroke in children: data from the nationwide inpatient sample. Stroke. 2007;38:1850–1854.
2. Nowak-Gottl, et al. Antithrombotic drug treatment of pediatric patients with ischemic stroke. Pediatric Drugs. 2003;5:167–175.
3. The International Stroke Trial (IST) A randomized trial of aspirin, subcutaneous heparin, both, or neither among 19435 patients with acute ischaemic stroke. International Stroke Trial Collaborative Group. Lancet. 1997;349:1569–1581.
4. National Institute of Neurological Disorders and Stroke rtPA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. N Engl J Med. 1995;333:1581–1587.
5. Hacke W, et al. Intravenous thrombolysis with recombinant tissue plasminogen activator for acute hemispheric stroke: the european cooperative acute stroke study. JAMA. 1995;274:1017–1025.
6. Multicentre Acute Stroke Trial Italy (MAST-I) Group. Randomised controlled trial of streptokinase, aspirin, and combination of both in treatment of acute ischaemic stroke. Lancet. 1995;346:1509–1514.

7. Williams GR. Incidence and characteristics of total stroke in the united states. BMC Neurol. 2001;1:2.

8. Lee BI, et al. Yonsei Stroke Registry. Analysis of 1,000 patients with acute cerebral infarctions. Cerebrovasc Dis. 2001;12:145–151.

9. Lee Y, et al. Risk factors and outcomes of childhood ischemic stroke in taiwan. Brain and Development. 2007;30:14–19.

10. Fullerton HJ, Wu YW, Zhao S, Johnston SC. Risk of stroke in children: ethnic and gender disparities. Neurology. 2003;61: 189–194.

11. Carpenter J, Tsuchida T, Lynch JK. Treatment of arterial ischemic stroke in children. Expert Review of Neurotherapeutics. 2007;7:383–394.

12. Shuayto MI, Lopez JI, Greiner F. Administration of intravenous tissue plasminogen activator in a pediatric patient with acute ischemic stroke. Journal of Child Neurology. 2006;21:604–606.

13. Roach SE, et al. Management of Stroke in Infants and Children: A Scientific Statement From a Special Writing Group of the American Heart Association Stroke Council and the Council on Cardiovascular Disease in the Young. Stroke. 2008;39:2644–2691.

14. Hurvitz E, et al. Long-term functional outcome of pediatric stroke survivors. Topics in Stroke Rehabilitation. 2004;11:51–59.

CASE REPORT

Adult Intussusception – A Diagnostic Dilemma.

KHANDUKER N[1], TARIQUE AA[2], AKHTAR G[3], KHANAM A[4], MD. RASHID MHO[5], CHOWDHURY NN[6], ALAM MM[7]

Abstract

Intussusception is invagination of one segment of the gastrointestinal tract and its mesentery (intussusceptum) into the lumen of an adjacent distal segment of the gastrointestinal tract (intussuscipiens). A 50 years old female patient presented with the complaints of abdominal pain for 1 month, intermittent vomiting for 20 days, constipation and abdominal distension for 10 days. She had history of similar attack repeatedly since last 3 months. On examination she was afebrile, mildly pale, abdomen was distended centrally, lower abdomen was tender and exacerbated bowel sound was audible. Per rectal digital examination was normal. MRI of abdomen revealed intussusception. Laparotomy was done which revealed ileoileal intussusception with an elongated polypoid lesion in the intestinal lumen. Reduction and end to end anastomosis was done. Adult intussusception is a rare condition. Because of its rarity and nonspecific symptoms and signs preoperative diagnosis is impossible. Laparotomy followed by surgical reduction is the treatment of choice as delay in diagnosis and treatment may lead to intestinal obstruction and ischemia.

Key words: Intussusception , Intestinal obstruction, Intestinal ischemia.

Journal of Green Life Med. Col. 2017; 2(1): 39-43

Introduction:

Adult intussusception is a rare condition which can occur in any site of gastrointestinal tract from stomach to rectum. It represents only about 5% of all intussusceptions[1] and causes 1-5% of all cases of intestinal obstruction[2]. Intussusception accounts for 0.003 – 0.02% of all hospital admissions.[3] The mean age for intussusception in adult is 50 years and male to female ratio is 1:1.3.[4] The child to adult ratio more than 20:1. The condition is found in less than 1 in 1300 abdominal operations and 1 in 100 patients operated for intestinal obstruction. Intussusception in adults occurs less frequently in the colon than in the small bowel.[5] Mortality for adult intussusception increases from 8.7% for the benign lesions to 52.4% for the malignant variety.[6]

1. Dr. Nabila Khanduker, Assistant Professor. Dept. of Surgery. Green Life Medical College.
2. Dr. Abdullah Al Tarique, Associate Professor, Dept of Surgery. Green Life Medical College.
3. Dr. Gulshan Akhtar. Associate Professor. Dept. of Paediatrics. Green Life Medical College.
4. Dr. Afroza Khanam. Assistant Professor. Dept. of Otolaryngology. Green Life Medical College.
5. Dr. Md. Haroon Or-Rashid. Residential Surgeon. Feni Sadar Hospital.
6. Dr. Nurun Nahar Chowdhury. Associate Professor & Head. Dept. of Psychiatry. Green Life Medical College.
7. Prof. Dr. Manjur Alam, Prof & Head, Dept of Surgery, Green Life Medical College

Address of Correspondence: Dr. Nabila Khanduker. Assistant Professor. Dept. of Surgery. Green Life Medical College. Email: nabilaarmin@gmail.com

Case presentation:

Mrs. Momtaz, 50 years old housewife hailing from Laxmipur was admitted with the complaints of:

1. Pain in the abdomen for 30 days
2. Intermittent vomiting for 20 days
3. Constipation and abdominal distension for 10 days.

She also gave history of recurrent attack of similar type of symptoms in last 3 months and was admitted at different hospitals repeatedly and treated conservatively followed by improvement and discharge.

This time on examination she was anxious, ill looking, dehydrated, hemodynamically stable, mildly pale and afebrile. There was central abdominal distension with lower abdominal tenderness and hyperactive bowel sound. Digital rectal examination was normal. Examination of other systems revealed no abnormality.

Plain x ray of abdomen showed multiple fluid gas level in the central portion of abdomen suggestive of small intestinal obstruction. USG of whole abdomen showed intestinal mass. MRI of abdomen was done which revealed intussusception.

Initially following admission she was treated conservatively by nothing per oral, nasogastric suction, intravenous fluid, intra venous broad spectrum antibiotics and anti ulcerants. Then she underwent exploratory laparotomy. At laparotomy, ileo-ileal intussusception was found. Reduction was done and an elongated polypoid lesion was found with in the lumen. The gut was viable. The affected segment with lesion was resected and gastro intestinal continuity was restored by end to end anastomosis.

Table-I

Lesions associated with adult intussusception. GIST- gastrointestinal stromal tumor

Lesion	Stomach	Small intestine	Large intestine
Benign	Adenoma	Lipoma	Lipoma
	Leiomyoma	Leiomyoma	Adenomatous polyp
	Lipoma	Hemangioma	Postoperative adhesion
	Hamartoma	Neurofibroma	Leiomyoma
	Inflammatory	Adenomatous polyp	GIST
	Polyps	Meckel's diverticulum	Endometriosis
		Intestinal duplication	Previous anastomosis
		Inflammatory lesion	Crohn's disease
		Trauma	
		GIST	
		Postoperative adhesions	
		HenochSchonleinpurpura	
		Tuberculosis	
		Giardiasis	
Malignant	Primary:	Primary:	
	Adenocarcinoma	Lymphoma	
	Leiomyosarcoma	Malignant duodenal ulcers	
		Malignant GIST	
		Secondary:	
		Metastatic melanoma	
		Adenocarcinoma metastasis (lung / breast)	
		Osteosarcoma	
		Lymphoma	
Idiopathic		Motility disorder	Motility disorder

Discussion:

Intussusception is defined as invagination of one segment of GIT and its mesentery into the lumen of adjacent distal segment of the GIT. Sliding with in the bowel is propelled by intestinal peristalsis and may lead to intestinal obstruction and ischemia. In adults intussusception has an identifiable etiology. Its etiology in different part of GIT is different. Fifty to seventy five per cent of adult small bowel intussusception are due to benign pathology.

The most common locations in the GIT where an intussusception can take place are the junctions between freely moving segments and retroperitoneally or adhesionally fixed segments. Stimulation of the GIT by food bolus produces an area of constriction above the bolus and relaxation below. Any intra luminal lesion in the GIT or irritant with in the lumen which alters the normal peristaltic pattern, is able to initiate intussusception. The stomach, duodenum and esophagus are rarely involved in intussusception because, they are less redundant and less mobile with in the abdomen.[7]

Classification of intussusception

There are no accepted classification of adult intussusception. One recommended classification is as follows:

1. The anatomic location of the intussusception:
   - gastroenteric
   - entero enteric
   - appendiceal
   - appendiceal – ileocolic
   - ileocolic
   - colocolic

2. According to etiology
   - 2.1: Neoplastic
     - Benign
     - Malignant
   - 2.2: Non neoplastic
   - 2.3: Idiopathic
3. According to lead point
   - Intussusception with lead point
   - Intussusception without lead point
4. According to clinical course
   - Acute
   - Chronic
   - Persistent
   - Recurrent
5. According to bowel obstruction
   - With lumen obstruction
   - Without lumen obstruction
6. According to vascular insufficiency
   - With disturbance of blood stream
   - Without disturbance of blood stream

Clinical presentation of adult intussusceptions:

Adult intussusception pose a further challenge as they are often presented with nonspecific symptoms and run a chronic indolent course. The spectrum of clinical presentation depends on the site of intussusception, the timing of clinical presentation and the predilection for spontaneous reduction. It may present as acute (duration less than 4 days), subacute (duration 4-14 days) and chronic (duration more than 14 days) or intermittent symptoms. Most patients manifest subacute (25%) or chronic (50-73%) symptoms.[8] Duration of symptoms is longer in benign lesions as compared with malignant lesion and is longer in enteric lesions as compared with colonic lesions.In adults intussusception typically manifests as acute or chronic obstruction and the presentation is similar to that of small and large bowel obstruction.

The most symptom in acute presentation is acute abdominal pain (71-100%) associated or not with an intestinal obstructive syndrome which occur in 78-100% of patients.[8,9,10] Intermittent abdominal pain and vomiting (40-60%) and or nausea are the major symptoms of subacute or chronic adult intussusception. Bleeding per rectum occurs in 8-27% of the cases (Table 2).

Table-II

Common clinical, physical and laboratory findings of adult intussusceptions

Clinical Presentation	
Abdominal pain	71 – 100%
Nausea	35 – 59%
Vomiting	31 – 59%
Loss of weight	4 – 33%
Episodes of diarrhea	9 – 28%
Hematochezia	8 – 27%
Constipation	13 – 26%
Fever	4 – 25%
Tenesmus	3%
Physical findings	
Abdominal distension	23 – 54%
Palpable abdominal mass	8 – 33%
Mass protruding through the anus	2 – 8%
Laboratory findings	
Anemia (Hb< 12 gm/dl)	43%
Leukocytosis	40%

Diagnostic tools for adult intussusceptions:

Preoperative diagnosis is a challenge because of rarity of adult intussusception, longstanding, intermittent, nonspecific symptoms and physical findings and signs on imaging. Despite of the evaluation of the radiological procedures, intussusception is diagnosed preoperatively from 14 to 75% of the cases. The most important factor in reaching at an accurate diagnosis are an awareness of the possibility of this condition existing in any patient with symptoms suggesting prior episodes of partial intestinal obstruction and vigorous approach towards complete radiographic examination in such patients[11]. Noninvasive radiologic imaging techniques can be of significant help in identifying an intussusception, but most cases are diagnosed at emergency operation, after abdominal exploration and excision of the intussuscepted segment of the GIT.

i) Diagnosis with plain abdominal film

It is the first diagnostic tool in acute abdomen and usually it demonstrates the signs of acute intestinal obstruction and sometimes provides information regarding to the site of obstruction.[12] Its sensitivity in intussusception is only about 25%.[13]

ii) Diagnosis with barium enema

Barium enema with barium reflux in the lumen of the space between the intussusceptum and intussuscepiens can help to identify the site and cause, particularly in chronic cases. Contrast studies are contraindicated if there is a possibility of bowel perforation and ischemia.

iii) Diagnosis with ultrasonography

USG is a useful tool for diagnosis of suspected case which shows characteristic doughnut sign in transverse section. It is quick, cost effective and shows when done by an experienced physician similar sensitivity and specificity like CT scan.[14]

iv) Diagnosis with computed tomography

Intussusception is well diagnosed on multi - slice spiral computed tomography with a diagnostic accuracy near 100%. Abdominal CT is the most useful diagnostic tool not only for detecting an intussusception but also helps in identifying the underlying cause.[15]

v) Diagnosis with magnetic resonance imaging

The imaging characteristic of an adult intussusception are similar to those on CT scan.

vi) Colonoscopy

It is also useful tool for evaluation of intussusception specially when the presenting symptoms indicate large intestinal obstruction but have limitation in small intestinal examination.

vii) Laparoscopy

Laparoscopy, although not an imaging study, is obviously an excellent evaluation tool when intussusception is suspected in a patient with bowel obstruction. It allows for identification of the location, the nature of the lead point and the presence of compromised bowel and helps in choice of an appropriate location for incision.[8]

Treatment:

Many therapeutic interventions have been tried for the treatment of adult intussusception which vary from conservative treatment to various surgical procedures. Treatment is almost always surgical in adults when compared to children and invariably leads to resection of the involved bowel segment with subsequent primary anastomosis. Overall the type of surgical intervention depends on the cause of intussusception (benign or malignant), patient's age, functional status, medical history and intra operative findings (a gangrenous bowel or a perforation with peritonitis; location and length of affected bowel segment). The main problem is to distinguish the benign and malignant lesions pre operatively.[16] Patients with malignant disease should undergo major surgery (ie; resection of involved segments and regional lymph nodes) while patients with benign lesions need simple resection.

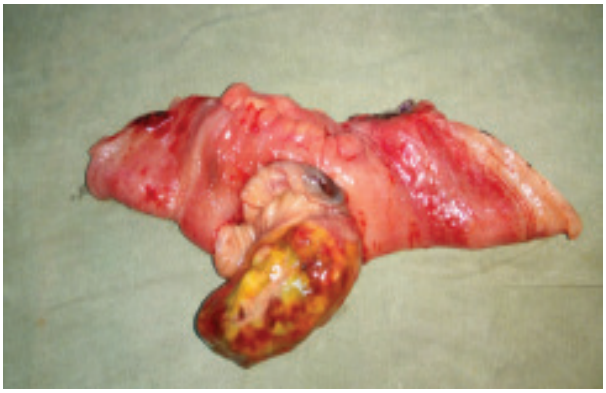


Fig.-1: *Resected portion of ileum containing inflammatory fibroid polyp*

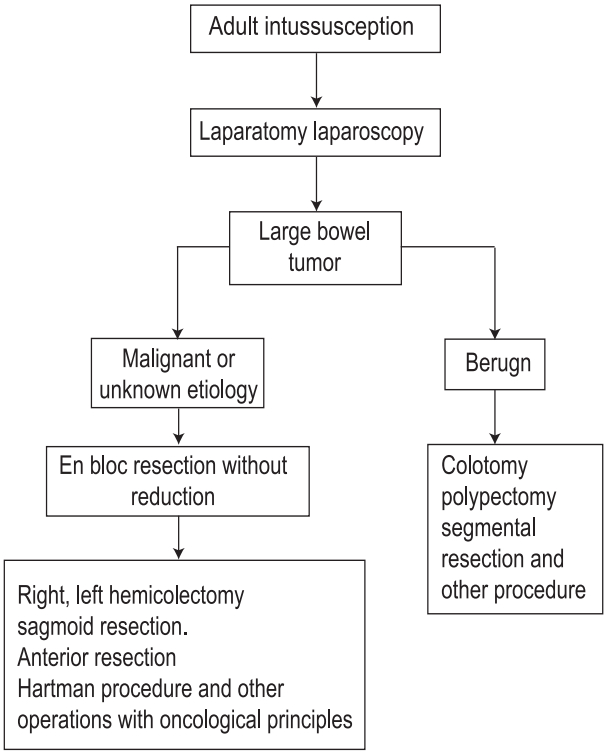


Fig.-2: *Algorithm of surgical treatment of adult large bowel intussusceptions with tumor*

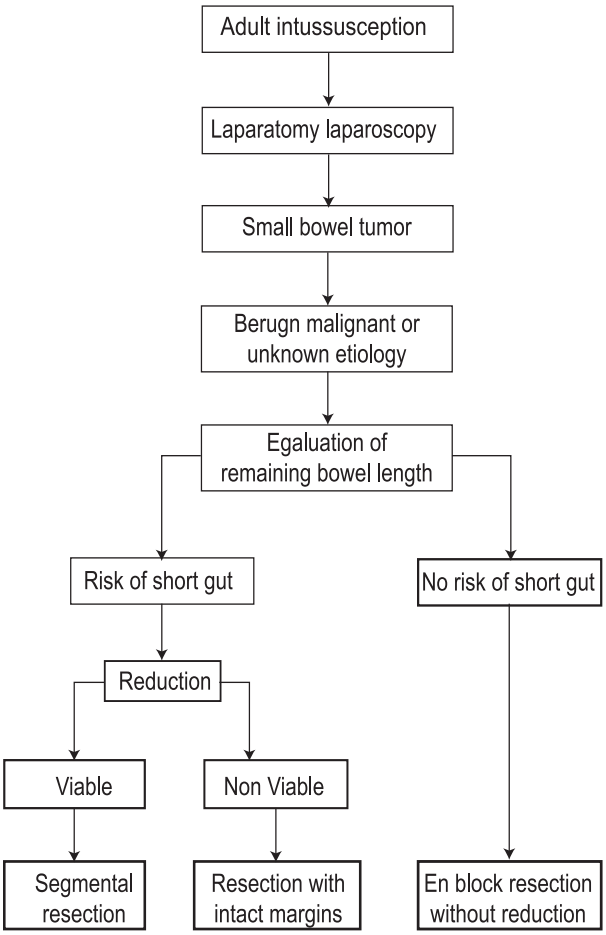


Fig.-3: *Algorithm of treatment of adult small bowel intussusceptions*

Prognosis and Complications:

Intussusceptions usually have good prognosis and depend on the cause. Mortality for adult intussusceptions increases from 8.7% due to benign lesions to 52.4% due to malignant causes.[17] Risk of mortality depends on bowel obstruction, complications, urgent operation, associated malignancy, but not on intussusceptions themselves. Most patients treated within the first 24 hours recover completely. Further delay increases the risk of complications, i.e. bowel ischemia, necrosis and perforation, infection and death. Recurrence after surgical treatment is rare condition.[8]

Conclusion:

Adult intussusception is a rare condition and can occur in any site of gastrointestinal tract from stomach to rectum. Because of rarity of this condition and nonspecific symptoms and physical finding, and signs on imaging, preoperative diagnosis is often difficult.

References:

1. Agha FP. (1986). Intussusception in adults. *AJR Am J Roentgenol.*, Mar 1986; 146(3): 527–31.
2. Begos DG, Sandor A andModlin IM. (1997). The diagnosis and management of adult intussusception. *Am J Surg.,* Feb 1997; 173(2): 88–89.
3. Weilbaecher D, Bolin JA, Hearn D and Ogden W. (1971). Intussusception in adults. Review of 160 cases. *Am J Surg.,* May 1971; 121(5):531–35.
4. Rathore MA, Andrabi SI and Mansha M. (2006). Adult intussusception - a surgical dilemma. *J Ayub Med Coll Abbottabad.,* Jul-Sep 2006; 18(3): 3–6.
5. Zubaidi A, Al-Saif F and Silverman R. (2006). Adult intussusception: a retrospective review. *Dis Colon Rectum.,*Oct 2006; 49(10):1546–51.
6. Azar T and Berger DL. (1997). Adult intussusception. *Ann Surg*., Aug 1997; 226(2):134–38.
7. Cera SM. (2008). Intestinal Intussusception. *Clin Colon Rectal Surg.,* May 2008; 21(2):106–13.
8. Barussaud M, Regenet N, Briennon X, de Kerviler B,.Pessaux P, Kohneh-Sharhi N, Lehur PA, Hamy A, Leborgne J, le Neel JC andMirallie E. (2006). Clinical spectrum and surgical approach of adult intussusceptions: a multicentric study. *Int J Colorectal Dis*., Jun 2005; 21(8): 834–39.
9. Erkan N, Hacýyanlý M, Yýldýrým M, Sayhan H, Vardar E &Polat AF. (2005). Intussusception in adults: an unusual and challenging condition for surgeons. *Int J Colorectal Dis.,*Sep 2005; 20(3): 452–56.
10. Paskauskas S, Latkauskas T, Valeikaitë G, Parseliûnas A, Svagzdys S, Saladzinskas Z, Tamelis A &Pavalkis D. (2010). Colonic intussusception caused by colonic lipoma: a case report. *Medicina*(Kaunas). 2010; 46(7):477-81.
11. Cotlar AM & Cohn I. (1961). Intussusception in adults. *Am J Surg.,* Jan 1961; 101: 114–20.
12. Eisen LK, Cunningham JD, Aufses AH Jr. (1999). Intussusception in adults: institutional review. *J Am Coll Surg*. Apr 1999; 188(4): 390-95.
13. Yakan S, Caliskan C, Makay O, Denecli AG &Korkut MA. (2009). Intussusception in adults: clinical characteristics, diagnosis and operative strategies. *World J Gastroenterol.*,Apr 2009; 28:1985-89.
14. Martin-Lorenzo JG, Torralba-Martinez A, Liron-Ruiz R, Flores-Pastor B, Miguel-Perelló J, Aguilar-Jimenez J & Aguayo-Albasini JL. (2004). Intestinal invagination in adults: preoperative diagnosis and management. *Int J Colorectal Dis.,* Jan 2004; 19(1): 68–72.
15. Huang BY &Warshauer DM. (2003). Adult intussusception: diagnosis and clinical relevance. *RadiolClin North Am* ., Nov 2003; 41(6): 1137–51.
16. Chiang JM & Lin YS. (2008). Tumor spectrum of adult intussusception. *J SurgOncol*., Nov 2008; 98(6):444-47.
17. Parashar UD, Holman RC, Cummings KC, Staggs NW, Curns AT, Zimmerman CM, Kaufman SF, Lewis JE, Vugia DJ, Powell KE, Glass RI. (2000). Trends in intussusception-associated hospitalizations and deaths among US infants. *Pediatrics.* Dec 2000; 106(6): 1413-21.

COLLEGE NEWS

Journal of Green Life Med. Col. 2017; 2(1): 44

- Council of Bangladesh College of Physicians and Surgeons (BCPS) has granted the recognition to the departments of Medicine, Obstetrics & Gynaecology and Orthopaedic Surgery of Green Life Medical College for imparting training to the resident doctors for a period of five (5) years with effect from January, 2017.
- Paper presented in CME/CPD organized by Medical Education Unit (MEU) of GMC from July to December, 2016:

Date	Topic	Organized by	Speaker
20.07.2016	How to make lecture effective	MEU	Dr. Lima Shompa
03.08.2016	Hair fall	Dept. Dermatology & Venereology	Dr. Mohammad Asifuzzaman
10.08.2016	Mystery of Pregnancy – "Hydatiform Mole"	Dept. of Obstetrics & Gynaecology	Dr. Lima Shompa
17.08.2016	Discussion on Updated Undergraduate (MBBS) Curriculum 2012	Dept. of Anatomy	Dr. Rumana Reza
28.09.2016	Primary open angle glaucoma	Dept. of Ophthalmology	Dr Muntaha Binte Rashid
05.10.2016	Gestational Diabetes Mellitus	Dept. of Endocrinology	Dr. Tanjina Hossain
09.10.2016	Hypertension	Dept. of Medicine	Dr. Ashif Al Mukit
02.11.2016	Rational use of antibiotics	Dept. of Pharmacology & Therapeutics	Dr. Md. Rifayet Rahman
16.11.2016	Sample collection,	Dept. of Biochemistry processing & forwarding	Dr. Sanjeela Nahreen Chowdhury
23.11.2016	Multiple skin cancer: tip of the iceberg	Dept. of Surgery	Dr. Nabila Khanduker
30.11.2016	Sustainable developmental goals: a challenge.	Dept. of Community Medicine	Dr. Sheela Khan
14.12.2016	When identity is in crisis	Dept. of Endocrinology	Dr. Tanjina Hossain
14.12.2016	Sound Noise and Noise Induced hearing loss	Dept. of ENT & Head-Neck Ssurgery	Prof. Dr. Pran Gopal Datta
21.12.2016	Gene therapy	Dept. of Microbiology	Dr. Bakibillah
28.12.2016	Osteoporosis: An unsolved problem	Dept. of Orthopedic Surgery	Dr. Pranab Kairy