C O N T E N T S

Journal, Indian Academy of Clinical Medicine • Vol. 11, Number 1, January – March, 2010

Contains pages from 1 to 80 inclusive of all advertisements

C O N T E N T S

Journal, Indian Academy of Clinical Medicine • Vol. 11, Number 1, January – March, 2010

Contains pages from 1 to 80 inclusive of all advertisements

Journal, Indian Academy of Clinical Medicine

JOURNAL, INDIAN ACADEMY OF CLINICAL MEDICINE

is edited by

BB Rewari

for the

Indian Association of Clinical Medicine

Headquarters :

Postgraduate Department of Medicine, SN Medical College, Agra – 282 002 (U.P.)

Editorial/Mailing Address

D II/65, Kaka Nagar, Dr. Zakir Hussain Marg, New Delhi – 110 003.
Tel.: (011) 2642 9099, 2352 5740 E-mail: sanjeev.initials@gmail.com, bbrewari@hotmail.com

Typesetting by: Initials, Tel.: 2361 7929, 2354 7929 E-mail: sanjeev.initials@gmail.com

ISSN 0972-3560

RNI Regn. No. : DELENG/2000/1686

Indexed in Elsevier's Bibliographic Databases

Indexed in IndMED (http://indmed.nic.in)

"Bibliographic details of the journal available in ICMR-NIC's database – IndMED (http://indmed.nic.in). Full-text of articles (from 2000 onwards) available on medIND database (http://medind.nic.in)."

Indexed Internationally on Elsevier's Bibliographic Databases (a leading indexing service) from January 2005. These databases include EMBASE, Compendex, Geobase and Scopus (Science Direct Navigator).

Printed by Dr. B.B. Rewari, Published by Dr. B.B. Rewari
on behalf of Indian Association of Clinical Medicine
and printed at Tan Prints, A-47, Mangolpuri Industrial Area, Phase II, Delhi,
and published from D II/65, Kaka Nagar, Dr. Zakir Hussain Marg, New Delhi – 110 003. Editor: Dr. B.B. Rewari

Indian Association of Clinical Medicine

GOVERNING BODY

President

G Narsimulu (Hyderabad)

President-Elect

Madhuchanda Kar (Kolkata)

Past-President

DK Hazra (Agra)

Vice-Presidents

PC Mathur (Gwalior)

Anil Chaturvedi (New Delhi)

Founder President

(Permanent Invitee)

MC Gupta (Agra)

Hony. Gen. Secretary

Ashok Shiromany (Agra)

Hony. Editor, *JIACM*

BB Rewari (New Delhi)

Associate Editor, *JIACM*

DG Jain (New Delhi)

Hony. Treasurer

Tejpal Singh (Agra)

Members

Brijesh Sharma (New Delhi)

MPS Chawla (New Delhi)

PK Bhattacharya (Darbanga)

Alladi Mohan (Tirupati)

Anita Sharma (Dehradun)

UN Gupta (Agra)

SK Saxena (Kanpur)

BK Agarwal (Mathura)

Zonal Members

North Zone

G Sidhu (Ludhiana)

South Zone

YS Raju (Hyderabad)

East Zone

BN Jha (Muzaffarpur)

West Zone

KK Pareek (Kota)

Central Zone

SK Verma (Dehradun)

Organising Secretary

(IACMCON' 2010)

Dipanjan Bandhopadhyay (Kolkata)

Organising Secretary

(IACMCON' 2009)

PK Jain (Jhansi)

Joint Secretaries

MN Rao (Hyderabad)

Prashant Prakash (Agra)

Alladi Mohan (Tirupati)

VIEWPOINT

JIACM 2010; 11(1): 12-5

Need-based Medical Education

BM Hegde*

"How much longer will medicine's flagship educational events fly the colours of the drug industry?"

– Ray Moynihan.

This is an attempt to look at our present day medical education from within, after nearly five decades of, I hope, fecund involvement. Modern medical education in India goes back to the year 1857, when the East India Company started three medical colleges – one each in Madras, Bombay, and Calcutta. The initial syllabus was brought from the London University. It has, of course, changed a bit here and there, but there has never been an attempt to really do some introspection for nearly one-century and a half. There has never been an attempt to see if our hoary past, with its wonderful medical knowledge, could, at least, be amalgamated with the western thoughts brought from outside, with benefit to the suffering humanity.

Most of us inside the system have a holier-than-thou attitude towards anything Indian that has not been certified to be scientific by the West. I think the time has come now to think of all that, as the West itself is looking to the East for inspiration in this field with the top heavy, hi-tech western medicine having become prohibitively expensive. The London Royal College of Physicians recently brought out a manual, following a symposium on *The Science of Alternative Medicine*, highlighting the positive aspects of the latter and also bringing-out a lot of good scientific material in them. It is time now for us to take a fresh look.

What is wrong with the present system?

On the surface everything looks good. Many would want to know that if it was good enough for us why not continue it for the next generation. How good is good? Modern hi-tech medicine, sold all over as very scientific has a very shaky foundation. David Eddy, a former associate professor of cardiovascular surgery at the Stanford University, USA, after very extensive research and doing his PhD in mathematics, wrote that 85% of what doctors do is based on very soft data, while only 15% is based on hard unequivocal data. He has now invented a new computer model – ARCHIMEDES – that shows most of our interventions in very poor light (www.archimedesmodel.com).

The recent UNIDO report showed that about 80% of the world's population today does not have the benefit of modern medicine. Many studies have very clearly shown that the most important risk factor for all diseases, from common cold to cancer, is poverty. Poverty and ignorance begin to have their ill effects on the future man right from the first trimester of pregnancy inside his mother's womb and bother him chasing him to the tomb! Studies in the West have shown that whereas the diseases are in plenty in the far-flung villages, surplus of doctors are in the cities and metropolises. This *inverse-care law*, propounded by a family doctor, Tudor Edward Hart, working in a Welsh mining village, speaks volumes about what happens in the third world countries.

The art of medicine, that which makes the patient's day, is not being taught enough in the medical schools. Even when passing remarks are made by some teachers about the art of medicine, most of it gets drowned in the sea of awe-inspiring technology. There is a lot we could do in this field, as shown very well by an Oxford professor, David Weatherall, in his book *The Science of Medicine and its Quiet Art*, or by Shervin B. Nuland, Stanford professor of clinical surgery, in his book *Wisdom of the Body*. These facts have been verified by a triple blind, computerised, prospective study in London, published in the *BMJ*, by the old students of Lord Platt, who now are the pillars of modern medicine in the UK. Professor Calnan's book *Talking with Patients* brings out the anguish of a thinking teacher at the

* Former Vice-Chancellor, Manipal University; Editor-in-Chief, The Journal of the Science of Healing Outcomes; Chairman, State Health Society's Expert Committee, Govt. of Bihar, Patna;
Visiting Professor of Cardiology, The Middlesex Hospital Medical School, University of London, U.K.;
Affiliate Professor of Human Health, Northern Colorado University, U.S.A.

Hammersmith hospital, London. Too much of technology and teaching subtleties to undergraduates were shown to be counter- productive in a study by three generations of teachers in the department of cardiology at the St. Andrews University, Dundee, UK.

The student gets lost in memorising data for the sake of the examination, and thus loses sight of the woods while counting the trees. Bereavement is an integral part of a doctor's life! Very little input is given in this direction to students to cope with it. Memorising the subject has another dangerous consequence in that the student, just before the examination, and if he passes the examination for ever after, deludes himself with the idea that he *knows everything that is to be known.* This feeling of "*knowing*" is suicidal in this field. One should aim at making the student realise genuinely that he *does not know!* That is where curiosity starts and wisdom begins. Another very important reason why medical education has become irrelevant to the present day needs is the craze for trying to do good to the *apparently healthy* in society in the name of *health screening* and *predicting the unpredictable*.

Predicting the future of any organism in this dynamic universe needs the knowledge of the *total initial state* of the organism, which is impossible today since we have no clue about the genotype of man and his consciousness! In addition, the linear thinking that if one were to change the *initial states* from abnormal to normal, there is no guarantee that this change holds good as time evolves. Time evolution in a dynamic system does not follow this rule. Long-term studies have shown the futility of this kind of exercise. Doctors have been *predicting the unpredictable* was the judgement of a physicist, professor Firth, in his enlightening article in the *BMJ* in 1991.

What could be done to rectify this?

Content and methodology

The syllabus is already overburdened. It cannot and should not be expanded; instead, it could be profitably cut short, without affecting the quality – nay even enhancing the quality of education. Problem-based learning, where the student and the tutor are both curious to learn, would be a better method. More time should be given to the students to think for themselves, in place of all the didactic teaching of facts. Facts keep changing everyday, what with the new information pouring in at a phenomenal pace of seven per cent per month. *The correct method of obtaining the data from many sources today is to be taught in place of teaching facts.*

Didactic lectures could be cut to the bare minimum, replaced by small group tutorials. Studies have shown that an unprepared mind absorbs less than five per cent of what is told in a lecture class. Clinical clerkships must take more of the student's time. There again, the ritualistic bedside clinics should give place to collective effort between the teacher and the taught to arrive at the diagnosis and management strategies for every patient under their charge. Then and then only, does the student realise the most important lesson in medicine that diagnoses and management are basically full of *uncertainty.* The grey zone in medicine is expanding every day, and the student should be aware of that as much as the teacher.

Medical education should be a collective effort at learning between the two parties, the teacher and the taught. The conventional *teaching by humiliation* should give place to learning with pleasure with a footing of equality. On the bedside, the student learns by observing the teacher, in all its ramifications, viz., manners, ready wit, compassion, understanding, human dignity, patient leeway, frustrations, anxieties, and what have you. This would give the student the courage to keep learning. To know that at times the *emperor also could be without his robes* is a very good stimulus to learn.

Specialists vs generalists

There was a craze for specialisation in the West for more than two decades now. There were so many specialities and subspecialities that they have now realised the bad effects of these both on the recipient, the patient, and also on the system. This kind of fragmentation is doomed to fail as per the 1st Law of Thermodynamics! Medical specialities grew directly proportionate to the growth of technology. The result is that technology has become top heavy and the hospitals have become prohibitively expensive even for the middle-class Americans.

The University of Minneapolis has started the system of

having three major specialities: general surgery, general medicine, and midwifery. Only when there is a definite indication for any type of intervention does the patient get referred to the particular specialist. A similar trend is coming to the UK also. Of course, we Indians believe in the dictum that we have to make the mistakes ourselves before we learn from them! We are wise enough not to learn from other's mistakes. We are where Americans were twenty years ago, starting more and more specialities and corporate hospitals.

A large country like ours, where more than 80% of patients are spread over the 5,75,000 odd villages, we would have to, per force, have more generalists. In addition, our present day medical training for a graduate is not conducive to send him to a village to manage alone. He would be a fish out of water there, as the ground realities in the community incidence of illnesses is not represented in the teaching hospitals. This is one of the main reasons why doctors do not want to go to the village. New doctors are not comfortable with their clinical abilities sans the hi-tech that they are used to in their medical school hospitals. Our graduates are good for working as junior doctors in larger corporate hospitals to order all the tests for every one who comes there for the boss to review or to work in junior posts abroad. Left alone in a village he would be helpless.

Evaluation

This is the real pain in the neck for both teachers and students alike. I know of no foolproof method of evaluation. The present system that we follow, which has been followed since the beginning of medical education in India, is far from satisfactory. Even though the best is yet to be thought of, we could try and make it more effective. The end-term, one-time examination should be replaced by continuous on-the-job-evaluation. This could be split into teacher evaluation and peer evaluation. *The latter could bring out the weaknesses and strengths of a candidate much more candidly.* The teacher evaluation should be a long drawn observation in place of the short, anxiety generating, incomplete assessment.

The debate about the type of theory examination is a never-ending one. The West went into the multiple choice objective theory tests, only to go back now to the time-tested essay type examination. However, both of them test the memory power of the examinee and not his total ability. In their place, a novel creative type of theory examination could be held. The student is posed a real life problem and is given enough time to write a critical answer on the lines of his future work outside. A critical appraisal of the problem should be able to give the candidate the capacity to learn the communication skills also in later life.

Practical and clinical examinations should mimic the real life situation. They should aim to assess the candidate's ability to listen to his patient, his compassion and human understanding, his knowledge of the clinical methods of eliciting the signs of disease, his interpersonal relations, his ability to get on with colleagues, his temperament as a doctor, and his mastery of the diagnostic skills and management strategies. Viva-voce examination is an opportunity to check the student's thinking capacity, instead of once again assessing his memory recall. This could be utilised to find out what kind of a doctor he would make in real life, his interest in furthering his skills and knowledge, his capacity to look at the same thing from different angles and also to fathom his reasoning power.

Examiners should have also a check on them. All the markings should be in the *close-marking method.* The positive and negative aspects of the student's abilities should be noted down for the future guidance of the candidate should s/he fail to make the grade. The examiners' performance should be computerised to assess them as examiners. Erring people should be blacklisted and their names sent to all the examining bodies with valid explanations. They could be reassessed after the lapse of a particular number of years!

Beyond the four walls of the classroom

Doctoring needs more skills than all that is written above. There are important areas not covered by the conventional teaching methods. One area that needs wider knowledge of human affairs is the capacity of the doctor to handle the only certain thing in life that is death. One of the questions asked is *why* did a patient get a particular disease or why did he die? These two questions could never be answered in biology. One

needs to know a bit of teleology and also philosophy. Positive sciences answer the question "how" or "how much", but not the question "why". One needs special skills of compassion and understanding to manage bereavement and separation.

The bane of modern medicine today is its cost. Every doctor must have an exposure to pharmaco-economics. It is one thing to read a book and write the medicine or order an operation, but the crux of the matter is if the recipient is able to afford that, and if not, what are the alternatives. That leaves the much-harried patient in a worse state. The mind of man is known to be the most important part of the whole gamut of health and disease, and the modern doctor should be able to unravel the depths of human mind, with a reasonable knowledge of human psychology, local customs, taboo, fears, anxieties and even superstitious beliefs. An assessment of the patient's surroundings, his worries, his anxieties, his near and dear ones, and his social ties would all have to be taken care of in some special situations.

Knowledge advances by refuting false dogmas. Genuine research demands that doctors keep an open mind on all aspects of their learning and try and get the false dogmas demolished to the extent possible. This requires the capacity to keep meticulous records of all our dealings with patients sincerely and honestly. Documentation should be taught to students from day one in their routine work as well. Research is not repeating others' work in your laboratory. *Clinical medical research is "having a question on the bedside and trying to go as far away from the bed as one could to get an answer."* We urgently need a uniform national standard for our postgraduate degrees to be recognised all over the world.

In short, medical education is an education for life. The right kind of education would bring out the best in every doctor who becomes patient friendly and would be able to do most good to most people most of the time. He is ideally *one who knows not, but, knows he knows not.* May his tribe increase!

"Men are vain authorities who could resolve nothing."

– Michel Eyquem de Montaigne (1533-92).

References

1 Moynihan R, Cassels A. Selling sickness: how drug companies are turning us all into patients. Sydney: Allen and Unwin, 2005.

2 Sorrell WH. Pharmaceutical marketing disclosures. publication date needed www.atg.state.vt.us/upload/1215544954_2008_Pharmaceutical_Marketing_Disclosures_Report.pdf

3 Moynihan R, Robertson J, Walkom E *et al*. A descriptive analysis of the world's first comprehensive disclosure of pharmaceutical industry funded events for health professionals. *Submitted to BMJ*.

4 Fletcher S. Continuing education in the health professions: improving healthcare through lifelong learning. Chairman's summary of the conference. Josiah Macy Jr Foundation, 2008. www.josiahmacyfoundation.org/documents/Macy_ContEd_1_7_08.pdf

5 Steinman MA, Bero LA, Chren M-M, Landefeld S. The promotion of Gabapentin: an analysis of internal industry documents. *Ann Intern Med* 2006; 145: 284-93.

6 Wazana A. Physicians and the pharmaceutical industry: is a gift ever just a gift? *JAMA* 2000; 283: 373-80.

7 American Association of Medical Colleges Task Force. https://services.aamc.org/Publications/index.cfm?fuseaction=Product.displayForm&prd_id=232&prv_id=281 (Industry funding of medical education).

P O E M

Greatest Surprise of Modern Times

Millions dying of heart, stroke and cancer caused by smoke,

Cursed by tobacco, quid, gutka and unknown spice,

Mouth filled with toxins feeling freshness and fragrance,

What an ignorance, self destruction, surprise and joke.

*– S Dwivedi**

**Professor and Head, Department of Medicine/Preventive Cardiology; Chief, Coronary Care Unit, University College of Medical Sciences, University of Delhi, GTB Hospital, Delhi*

VIEWPOINT

JIACM 2010; 11(1): 16-9

Chronic Diseases and the Medical Science: Urgent Need for a Paradigm Shift!

*BM Hegde**

There seems to be a crisis in modern medical research. While the modern medical research, based on statistics, is expanding exponentially straining the budgets of even the most affluent countries, the problems of chronic diseases like diabetes, atherosclerotic diseases, Parkinson's disease, Alzheimer's, and cancer are not being solved. Rather, their incidence has been stationary for well over one hundred years although we are made to believe that they are going up! While there seems to be a relative increase in the incidence, there is no absolute increase in any of these diseases. The former is due to wrong labelling methods as also to awareness in society to seek medical attention sooner than warranted, thanks to the screening industry. The problem with modern medicine is that it is still lost in the reductionist concept of organ-based diseases resting on the two pillars of anatomic paradigm of Vesalius (450-years-old) and the Mendelian paradigm of genetics.

When more and more research becomes unproductive, problems get more complicated. It is then necessary to change the paradigm, according to Thomas Kuhn, writing in his book *The Structure of Scientific Revolutions*[1]. Kuhn feels that the hypothesis-based research fails miserably in this scenario. Our concept of organ-based diseases and the development of subspecialties like cardiology, neurology, gastroenterology, etc., has been the major blow to productive research based on holism, which looks at the whole organism, its surroundings, as also its evolution. We need to have a total paradigm shift if we need to unravel the mysteries of ageing and chronic diseases that go with ageing. We could still keep the useful parts of the old paradigm like emergency procedures and surgical corrections in some settings along with the new paradigm. Time has come to say goodbye to disease-based research in preference to causation-based research.

"First, human diseases affecting a wide range of organs could result from systemic defects in energy metabolism and, second, hereditary human diseases could result from mutations in the non-Mendelian mtDNA. Consequently, mitochondrial biology and genetics become excellent candidates for expanding the anatomical and Mendelian paradigms to address the complexities of the age-related diseases, ageing, and cancer"[2]. This brings us to the original energy-based medical physiology and the non-Mendelian genetic inheritance as well.

"Life involves the interplay between structure and energy. For the eukaryotic cell, this duality was cemented ~ 2 billion years ago by the symbiosis of what appears to have been a glycolytic motile cell, which gave rise to the nucleus-cytosol, and an oxidative α-proteobacterium, which evolved into the mitochondrion[3]. Initially, each organism was free living and contained all of the genes for an independent life form. However, over the subsequent 1.2 billion years, the single-cell descendants of the initial symbiosis experimented with many alternative arrangements of biochemical interdependence and genomic reorganisation. Ultimately, however, an arrangement was achieved in which the mitochondrion became specialised in energy production and the nucleus – cytosol became specialised in structure. This final design provided the impetus for the development of multicellularity and the evolution of higher plants and animals, including humans"[4].

Let us first examine this nature's computer inside every cell which existed for nearly a billion-and-half years before the first nerves ever appeared in Jellyfish. It took another half-a-billion years for cranial nerves and brain to appear in organisms including man. The myth that the

* *Former Vice-Chancellor, Manipal University; Editor-in-Chief, The Journal of the Science of Healing Outcomes; Chairman, State Health Society's Expert Committee, Govt. of Bihar, Patna; Visiting Professor of Cardiology, The Middlesex Hospital Medical School, University of London, U.K.; Affiliate Professor of Human Health, Northern Colorado University, U.S.A.*

brain and the nervous systems alone keep us going has to give place to a more holistic view that there are many other important systems that help keep man alive. Then we will progress further in our understanding of human physiology and pathology. Life is a complicated system of individual cell function in the body in an interdependent manner to keep us alive and healthy. If the ten thousand odd proteins (part of the mitochondrion-based energy producers) that are present inside each one of the trillions of cells in our bodies do not work well we will have disease states. For recovery from any illness, body cells will have to function normally again[5].

Our approach in the "so-called" evidence-based modern medicine is to try and correct those changes (not knowing what they are) in disease states using chemicals or surgery. Chemicals try to change the nucleus cytosol system, hereinafter called the hardware of the computer that is the human cell, which is not conducive to healthy physiology. Surgery is useful in anatomic abnormalities but has a very limited role in health restoration. As a quick-fix, apparently, chemicals and surgery help some people sometimes but all of them damage some part of the human system almost always, sometimes as late as five years after the event. One good example is a pain killer trasylol which is now known to kill the recipient as late as five years after he/she had it! What happened to another pain killer wyoxx is now common knowledge. These measures have now resulted in considerable misery for mankind. To quote the most authentic scientific body of the USA: "The National Academy's data attributes 100,000 deaths per year to physicians' errors, added to well over 100,000 deaths due to severe drug interactions and another 100,000 fatalities from hospital-based-infections. (For a detailed analysis, see *Death by Medicine*, by Gary S Null *et al*)". This is from a country with less than one-third the population of India. Thank God, we do not have statistics like this for India[6].

What is the remedy? We must get to know the true physiology of cell function and try and see how we could restore that in the unlikely event of disease in a more natural way rather than inflicting chemical and surgical damage to the cells where possible. The fruitless research of modern medicine is based on statistical science and not true hard science. The "failure of millions of dollars spent on AIDS vaccine, failure of interferon as a wonder drug for cancer management with the latter still eluding a cure despite billions being wasted on cancer research and cancer screening (the latter has been shown to be useless and dangerous), and the ravages caused by drugs like thalidomide and thorazine", are there for all of us to see.

We need to arrange an urgent marriage between the beneficial remedies in modern medicine "like the excellent emergency care methods, brilliant surgical successes, time tested and harmless pharmaceuticals as also the newer lifestyle changes" with the best and scientifically authenticated multitude of methods in many other systems of medicine into a judicious integrated system of medical care that is inexpensive, safe, and effective under all circumstances. Unfortunately, the vested interests in modern medicine are scuttling every effort in this field by hitting those efforts with an old but, effective whip "there is no evidence base" in other systems of medical care. This is the biggest lie in the world.

Let us examine how we can use natural methods to get the damaged cells back to normalcy. The ten thousand odd proteins (part of the software) in each cell are functionally better than our supercomputers. They have two energy systems – the low energy system and the high energy system. Initially, the proteins process all the information they collect from the body as also the outside world into a low energy *information system* which primes the other proteins to a high energy *functional system* that could power the body as a whole. In this milieu there are certain specific proteins that do the directing or chaperoning job very effectively. One such chaperone protein is the Heat Shock Protein, HSP 70. It is otherwise called Stress Responsive Protein (SRP 70) as it responds to every kind of stress in the cells[7].

The HSP 70 protein is supervised by the HSP 70 gene. HSP 70 protein could be re-primed by heating the cell to 47 degree centigrade but, that can never be done in the human body. The other method is to use some kind of natural energy to do the job. In health, the cell uses the energy coming from the main source, sunlight, as also the magnetic energy generated by lightening throwing a halo of Schumann energy field around the earth (Schumann

effect) since all proteins are but carbon, hydrogen, nitrogen, and oxygen as in the DNA, along with amino-acids that come from food. The electromagnetic energy used by the cell proteins which is then transduced to fire the mitochondria inside every cell to produce energy needed for life[8].

Glen Gordon was one of NIH's brilliant young scientists, 4th in hierarchy at one stage. He was a pioneer in this field of trying to regenerate the damaged (ischaemic) cells back to normal. This made the American Medical Association to file a law suit against him, which did not materialise at the end. He lost his entire grant support, though. He would not relent. He has come-up with a small Pulsed Electro-Magnetic Field (PEMF) generator powered by a battery to stimulate and up-regulate the depressed HSP 70 protein and thereby regenerate the cells again. My own initial enthusiasm with this toy of his is exciting. This is not the right forum to disclose the data of our pilot study which is ongoing. None the less, the results are an opening for us to look more deeply into many such natural methods of making the sick cells (individuals) to regain their strength and health without any long-term detriment to the owner in the bargain[9, 10, 11].

The relationship between Chi energy and mitochondrial energy systems was brought out in an excellent article by Douglas C Wallace in the journal *Genetics* June 2008 issue is a revolutionary idea. Wallace states: "If this strategy proves successful, then it may have been prescient that a major concept in the parlance of traditional Asian medicine is 'chi', which loosely translates as "vital force or energy[12]". This is what is called *praana* in Indian ayurveda and yoga.

These specific mitochondrial genomic concepts provide a plausible explanation for the predisposition toward and development of age-related diseases. The mitochondrial energetic capacity might have an initial capacity at birth based on inherited nuclear DNA and mtDNA, the encoded mitochondrial genes. This could modulate the mitochondrial energetic output, antioxidant defenses, apoptotic thresholds, mitochondrial biogenesis and turnover, etc. As age advances, somatic mtDNA mutations arise in post-mitotic cells and stem cells[13-21]. When the mutant mtDNA accumulates in ageing cells, they erode into the cellular energy systems leading to decline in organ function, loss of cell numbers, tissue failure, and ageing.

Whereas mitochondrial damage is systemic, clinical manifestations might be organ specific as some organs like the retina, cochlea, some parts of the CNS, the heart, the muscles, and the kidney might suffer faster than the rest of the body parts. Some tissues store energy in their fat and suffer less. Liver maintains energy homoeostasis maintaining the serum glucose level within acceptable limits. The α- and β-cells in the pancreas could sense energy needs by monitoring calorie type and availability to send appropriate signals to glucagon or insulin, "to the energy-utilising, storage, and homoeostasis tissues[4]."

Our basic lack of knowledge of the mitochondrial biology limits our conventional reductionist research options in therapeutics. Wallace and colleagues feel our best bet would be to go to traditional Asian herbs that have been tested by trial and error to have the healing outcome effects. This could give us a jump start in this area. To detect mitochondrially active compounds among the multitude of Asian herbal drugs, they have developed a mitochondrially active cDNA expression array, "the MICTOCHIP that interrogates 1,000 genes involved in mitochondrial energy production, ROS biology, and apoptosis". They have found out that *Ginkgo biloba* leaf extract and *G. biloba* for on mitochondrial function in cultured cells. They did find those did indeed alter mitochondrial gene expression to modulate apoptosis[22]. "The association between Asian herbal medications and mitochondria has been further enhanced by the discovery that the potent antimalarial Artemisinin (quinghaosu) acts on the mitochondrion[23], and the observation that, after screening 2,490 compounds for the effects on mitochondrial gene expression and physiology, the Chinese herbal derivative deoxysppanone B was found to act through microtubules to increase OXPHOS and decrease mitochondrial ROS[24]."

Perhaps, then, a systematic survey of Asian herbal medications using a variety of mitochondrial functional readouts may reveal previously unrecognised mitochondrial pathways and new therapeutic strategies to manipulate them. These could then be applied to treating the common age-related diseases. This discovery clubbed with Glen Gordon's seminal studies on the pulsed electromagnetic energy supply to the

damaged or ageing cells might open up a new vista for holistic research for the future good of mankind. Here again the thrust must be to assess the healing outcomes and not the time-honored RCTs of modern medical statistical science. Our group has started a new journal, *Journal of the Science of Healing Outcomes* (www.thejsho.com) that specifically publishes super peer reviewed articles on the science of healing outcomes. Chronic disease scenario needs a specialty of its own with a paradigm shift[25].

References

1. Kuhn TS. The Structure of Scientific Revolutions. Chicago, University of Chicago Press, 1996.
2. Wallace DC. Mitochondrial genetics: A paradigm for aging and degenerative diseases? *Science* 1992; 256: 628-32.
3. Margulis L. Symbiosis in Cell Evolution: Life and Its Environment on the Early Earth. San Francisco, WH Freeman, 1981.
4. Lang BF, Burger G, O'Kelly CJ *et al*. An ancestral mitochondrial DNA resembling a eubacterial genome in miniature. *Nature* 1997; 387: 493-7.
5. Gray MW, Burger G, Lang BF. Mitochondrial evolution. *Science* 1999; 283: 1476-81.
6. Wallace DC. Why do we still have a maternally inherited mitochondrial DNA? Insights from evolutionary medicine. *Annu Rev Biochem* 2007; 76: 781-821.
7. Fried S. Bitter Pills: Inside The Hazardous World of Legal Drugs. New York. Bantam Books 1998.
8. Gordon GA. Extrinsic Electromagnetic Fields, Low Frequency (Phonon) Vibrations, and Control of Cell Function: A Non-linear Resonance System. *J Biomed Sci and Engineer* 2008; 1: 152-6.
9. George I, Geddis MS, Lill Z *et al*. Myocardial function improved by electromagnetic fled induction of stress protein HSP 70. *J Cell Physiol* 2008; 216 (3): 816-23.
10. Goodwin T. Physiologic and Molecular Genetic Effects of Time Varying Electromagnetic Fields on Human Neuronal Cells. NASA TP 2003/212054, NASA Technical and Scientific Library, Johnson Space Center, Houston TX.
11. Hegde BM. Is the Heart a Simple Muscular Pump? *J Ind Acad Clinical Med* 2008; 9: 172-4.
12. Grant G, Cadossi R, Steinberg G. Protection against Focal Cerebral Ischaemia Following Exposure to a Pulsed Electromagnetic Field. *Bioelectromagnetics* 1994; 3 (15): 205-16.
13. Wallace DC. Diseases of the mitochondrial DNA. *Annu Rev Biochem* 1992; 61: 1175-1212.
14. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet* 2005; 39: 359-407.
15. Wallace DC, Lott. MT, Procanno V. Mitochondrial genes in degenerative diseases, cancer and aging, 2007; pp. 194-298 in *Emery and Rimoin's Principles and Practice of Medical Genetics*, Ed. 5, edited by Rimoin DL, Connor JM, R Pyeritz E, Korf BR, Churchill Livingstone Elsevier, Philadelphia.
16. Muller-Hocker J, Seibel P, Schneiderbanger K, Kadenbach B. Different in situ hybridisation patterns of mitochondrial DNA in cytochrome c oxidase-deficient extraocular muscle fibres in the elderly. *Virchows Arch A Pathol Anat Histopathology* 1993; 422: 7-15.
17. Khrapko K, Bodyak N, Thilly WG *et al*. Cell-by-cell scanning of whole mitochondrial genomes in aged human heart reveals a significant fraction of myocytes with clonally expanded deletions. *Nucleic Acids Res* 1999; 27: 2434-41.
18. Wang Y, Michikawa Y, Mallidis C *et al*. Muscle-specific mutations accumulate with aging in critical human mtDNA control sites for replication. *Proc Natl Acad Sci USA* 2001; 98: 4022-7.
19. Herbst A, Pak JW, D McKenzie D *et al*. Accumulation of mitochondrial DNA deletion mutations in aged muscle fibers: evidence for a causal role in muscle fiber loss. *J Gerontology A Biol Sci Med Sci* 2007; 62: 235-45.
20. Michikawa Y, Mazzucchelli F, Bresolin N *et al*. Aging-dependent large accumulation of point mutations in the human mtDNA control region for replication. *Science* 1999; 286: 774-9.
21. McDonald SA, Preston SL, Greaves LC *et al*. Clonal expansion in the human gut: mitochondrial DNA mutations show us the way. *Cell Cycle* 2006; 5: 808-11.
22. Smith JV, Burdick AJ, Golik P *et al*. Anti-apoptotic properties of Ginkgo biloba extract EGb 761 in differentiated PC12 cells. *Cell Mol Biol* 2002; 48: 699-707.
23. Li W, Mo W, Shen D *et al*. Yeast model uncovers dual roles of mitochondria in action of artemisinin. *PLoS Genet* 2005; 1: e36.
24. Wagner BK, Kitami J, Gilbert TJ *et al*. Large-scale chemical dissection of mitochondrial function. *Nat Biotechnology* 2008; 26: 345-51.
25. Hegde BM. Eye of the beholder-Boondoggle? http://bmj.com/cgi/eletters/338/may21_1/b2068#214195, 23 May 2009.

ORIGINAL ARTICLE

JIACM 2010; 11(1) : 20-5

Assessment of Oxidative Stress in Patients of Diabetes Mellitus with and without Complications

Biplab Mandal, Ramtanu Bandyopadhyay**, Srabani Chakrabarti***, Tushar Kanti Bandyopadhyay*****

Abstract

Introduction: Oxidative stress plays an important role in different disease processes. Association of diabetes mellitus and its complications with oxidative stress has been demonstrated in experimental studies on animals. Some studies conducted on diabetic patients also support it. But very few studies have been conducted in the Indian subcontinent so far. This study was designed to assess the levels of oxidative stress in patients suffering from diabetes mellitus and to compare them with controls and also to correlate these levels of oxidative stress in cases of diabetes mellitus with and without complications.

Materials and methods: Thirty-three diabetic patients were divided into two groups. Group A comprised of 15 (fifteen) diabetic patient without complications and group B comprised of 18 (eighteen) diabetic patients with complications. Thirty normal healthy persons were selected for the study to serve as control. Serum MDA level which is a product of lipid peroxidation and vitamin A level which acts as anti-oxidant were estimated in all diabetic patients including controls.

Results: Serum MDA levels were significantly high in diabetic patients in comparison to normal individuals (6.14 ± 0.50 micromols/l vs 4.40 ± 0.17 micromoles/l and p < 0.001). When compared the MDA levels were much higher in patients with diabetic complications than without complications (6.57 ± 0.22 micromoles/l vs. 5.63 ± 0.16 and p < 0.001).

Vitamin E, which acts as an antioxidant was significantly higher in normal individuals in comparison to diabetic patients (11.48 ± 0.35 mg/l vs. 7.97 ± 0.62 mg/l and p < 0.001). It was also observed that Vit E levels were significantly high in diabetic patients without complications than in those with complications (8.62 ± 0.25 mg/l vs. 7.43 ± 0.13 mg/l and p < 0.001).

Conclusion: The present study concludes that diabetic patients suffer from more oxidative stress. Oxidative stress is higher in diabetic patients with complications than those without complications. Oxidative stress plays a significant role in diabetes mellitus and complications.

Key words: Diabetes mellitus; Oxidative stress; Serum MDA and Vit E levels.

Introduction

Diabetic complications account for much of the morbidity and mortality in diabetes. Although the Diabetes Control and Complications Trial (DCCT) has identified hyperglycaemia as a risk factor for development of diabetic complications, there is no consensus regarding the pathogenic link between hyperglycaemia and complications. In the last decade, oxidative stress related to increased production of free radicals and/or to antioxidant depletion has been implicated strongly in favour of the pathogenesis of diabetes mellitus and its complications.

'Oxidative stress' defined as an imbalance between ROS (reactive oxygen species) and cellular antioxidant defence system[1,2] is responsible for various pathogenetic processes. Oxidative stress may cause cellular dysfunction by promoting formation or AGEs, by inducing DNA strand breaks and activating poly ADP-ribose polymerase (PARP)[3,4], by causing dysfunction of eNOS, and by activating P38 and other stress-activated pathways leading to apoptosis.

Considerable experimental studies on animals for association of increased oxidative stress in diabetes mellitus and its complication had already been conducted. Also some studies conducted abroad show increased levels of oxidative by-products and decreased levels of antioxidants in patients with diabetes mellitus with and without complications. But few studies have been conducted in India so far. Paucity of Indian literature formed the basis of the present study, which was planned with the following aims and objectives:-

1. To study the levels of oxidative stress in patients

** Department of Medicine, Institution Medical College, Kolkata - 700 073, West Bengal.*

suffering from diabetes mellitus and to compare them with controls.

2. To correlate this levels of oxidative stress in cases of diabetes mellitus with and without complications.

Materials and methods

Study design: case control study

This study was carried out in the Department of Medicine, Medical College, Kolkata. Thirty-three patients who either attended the diabetic clinic or were admitted in the department and suffering from diabetes mellitus with or without complications were taken-up for the present study. Properly age and sex matched thirty normal healthy subjects (either patients' attendants or college staff) were selected in the present study to serve as controls.

Each case was evaluated with detailed history regarding the age of onset of diabetes, level of control of blood glucose. A particular stress was given to elicit the clinical symptoms of neuropathy, nephropathy, and retinopathy.

Each case was subjected to a thorough physical examination and detailed assessment as regards to neurologic and renal status. Detailed examination of the fundus was carried-out in each case to assess the retinopathic changes.

The routine investigations carried-out in each case included complete haemogram, urine analysis for proteinuria, glycosuria, ketonuria and microscopic sediments, blood urea, serum creatinine, blood sugar fasting and 2 hour after 75 gm of glucose load. HbA1c levels were estimated in each case to asses the status of control of diabetes.

Specific investigations like microalbuminuria, fundus photography/fluorescein angiography and nerve conduction studies was carried-out to establish the diagnosis of nephropathy, retinopathy, and neuropathy.

Exclusion criteria

Smokers and patients taking vitamin E or any anti-oxidant preparation were excluded from this study.

All the diabetic patients were divided into two groups:

A. Diabetics without complications.

B. Diabetics with complications.

Each diabetic case with or without complications was subjected to the following tests for assessing the oxidative stress and antioxidant level:

1. MDA levels in serum by thiobarbituric acid test,

2. Vit E level in serum.

Observations

A total of thirty-three (33) patients consisting of 18 male and 15 female patients suffering from diabetes mellitus were recruited to the study. The patients were subdivided into two groups:

1. Group A – Diabetes mellitus without complication (n – 15), which comprised of 8 males and 7 females.

2. Group B – Diabetes mellitus with complications (n – 18), which comprised of 10 males and 8 females.

Thirty normal healthy subjects comprising 16 males and 14 females with comparable age were selected for the control group in our study.

Patient profile

Table I shows patients' profile, with duration of diabetes and their fasting and post-prandial blood sugar levels.

Table I: Patients' profile.

	Diabetics with complications (n=18)	Diabetics without complications (n=15)
Male : female	10:8	8:7
(Age in years)	48.72 ± 9.93 (25-65)	45.13 ± 16.77 (17-68)
Duration of diabetes (in yrs)	7.17 ± 6.24 (0.5-24)	2.87 ± 2.63 (0.08-13)
Fasting blood sugar (mg/dl)	196.17 ± 59.70 (115-310)	157.73 ± 27.06 (120-220)
Post-prandial blood sugar (mg/dl)	328.06 + 67.74 (240-488)	260.67 ± 85.62 (160-300)

Assessment of oxidative stress

Serum MDA levels were estimated, which is a product of lipid peroxidation formed as a result of oxidative stress.

Serum levels of MDA increase proportionately with the levels of oxidative stress.

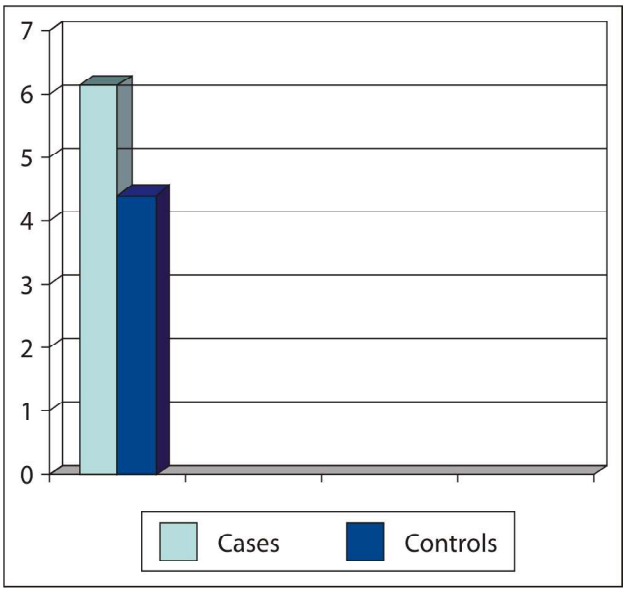


Fig. 1: *Bar diagram showing the levels of serum MDA in cases and in control group.*

Table II illustrates comparison of MDA levels in serum between cases of diabetes mellitus and controls.

Table II: Serum MDA levels in cases of diabetes mellitus and in controls.

Blood levels	Cases n=33	Control n=30	p-value
MDA level (micromol/l)	6.14±0.50 (5.20–6.85)	4.40±0.17 (3.00–3.70)	<0.001

MDA levels were 6.14 ± 0.50 (5.20 – 6.85) in cases of diabetes mellitus, while it was 3.40 ± 0.17 (3.00 – 3.70) in normal subjects. Serum MDA levels were significantly increased (< 0.001) in diabetic subjects.

Table III shows comparative analysis of serum MDA levels between diabetics without complications and those with complication.

Table III: Serum MDA levels in diabetic patients with and without complications.

Blood levels	Diabetics without complications (n=15)	Diabetics with complications (n=18)	p-value
MDA level (micromol/l)	5.63±0.16 (5.20–5.78)	6.57±0.22 (6.20–7.00)	<0.001

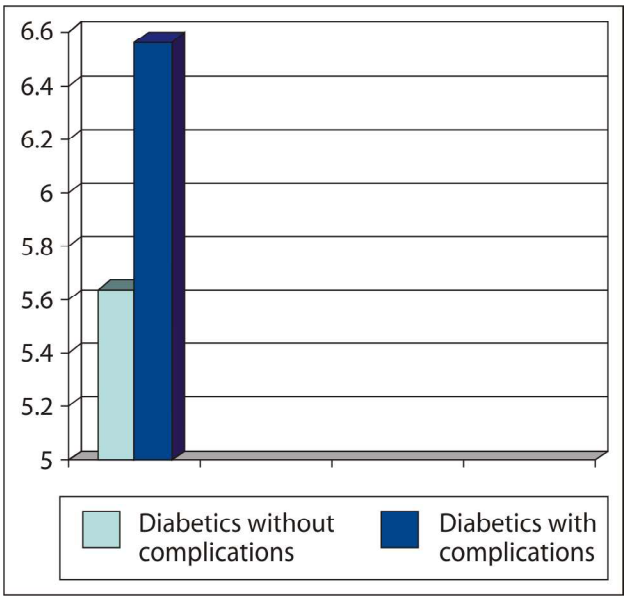


Fig. 2: *Bar diagram showing the comparison of MDA level among diabetic patients with and without complications.*

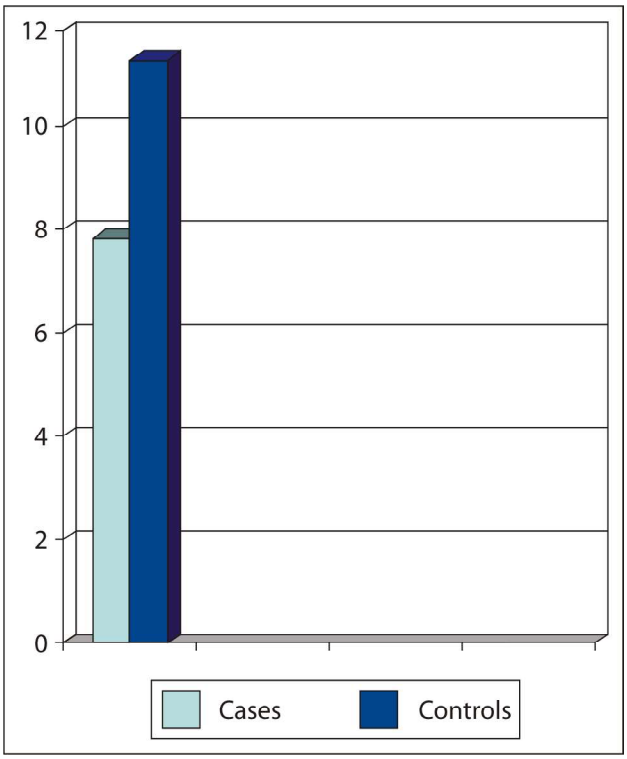


Fig. 3: *Bar diagram showing the comparison of serum vit-E level in both groups (cases and controls).*

Mean serum MDA levels were 5.63 micromol/l (SD 0.16) in diabetics without complications, and were 6.57 micromol/l (SD 0.22) in diabetics with complications. MDA levels in

diabetics with complications were significantly raised ($p < 0.001$) in comparison to diabetics without complications.

Serum level of vit. E was also estimated which acts as an antioxidant and protects the tissues from the ill-effects of oxidative stress. Decreased levels of serum vit E suggests increased oxidative stress and vice versa.

Table IV shows the comparative data of vitamin E levels in the serum between cases of diabetes mellitus and controls.

Table IV: Serum vit. E levels of cases of diabetes mellitus and controls.

Blood levels	Cases n=33	Control n=30	p-value
Serum-vit E levels (mg/l)	7.97±0.62 (7.20–9.00)	11.48+0.35 (10.70–12.00)	<0.001

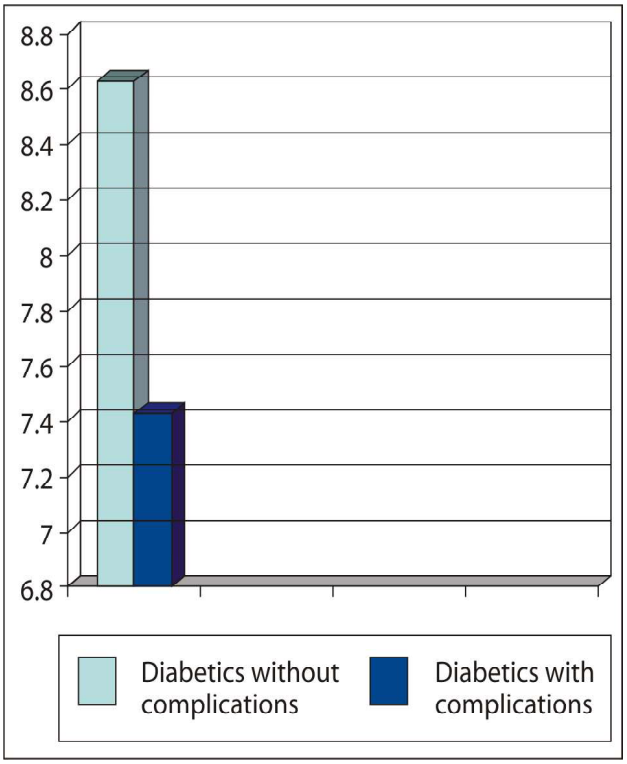


Fig. 4: *Bar diagram showing the comparison of vit-E level among diabetic patients with and without complications.*

Serum vitamin E level was 7.97 mg/l (SD 0.62) in cases of diabetes mellitus while it was 11.48 mg/l (SD 0.35) in control subjects. This decrease in levels of vit. E in cases of diabetes mellitus was highly significant (< 0.001). It suggests that they have suffered more oxidative stress than control subjects.

Table V shows comparative analysis of serum vit. E levels in cases of diabetes mellitus with and without complication.

Table V: Serum vit. E levels in diabetes with and without complications groups.

Blood levels	Diabetics without complications (n=15)	Diabetics with complications (n=18)	p-value
Vit-E level (mg/l)	8.62±0.25 (8.10–9.00)	7.43±0.13 (7.20–7.70)	<0.001

In diabetics without complications, mean vit. E level was 8.62 mg/l (SD 0.25) while it was 7.43 mg/l (SD 0.13) in diabetics with complications. When compared it was statistically significant ($p < 0.001$). Decreased levels of vit E, which is a natural anti-oxidant in our study, suggests that oxidative stress was higher in diabetic complications in comparison to diabetics without complications.

Discussion

In the present study, estimation of serum MDA (malondialdehyde) which is formed as a result of lipid peroxidation, and serum vit E which is a part of antioxidant system, were done.

In our study serum MDA levels were estimated in cases of diabetes mellitus and control subjects to compare the amount of oxidative stress between them. Serum levels of MDA were 614 micromoles/l (SD 0.50) in diabetic subjects whereas these were 3.40 micromoles/l (SD 0.17) in normal healthy subjects. Significantly higher levels of MDA were observed ($p < 0.001$) in diabetic persons in comparison to healthy normal controls.

Comparative evaluation of the serum levels of MDA was done in patients of diabetes mellitus with and without complications. It was 6.57 micromol/l (SD 0.22) and 5.63 micromol/l (SD 0.16). This difference was highly significant ($p < 0.001$).

Increased levels of serum MDA in cases of diabetes mellitus with complications in comparison to cases of diabetes mellitus without complications indicate more association of oxidative stress with diabetic complications.

Oxidative stresses in diabetics with or without vascular complications were evaluated in an interesting study by Sainani and Bambolkar[5]. In their study they compared the MDA level in diabetic patients without complications which was 5.38 ± 1.015 micromol/l and was 3.343 ± 0.73 micromol/l in control subjects. This difference was also highly significant ($p < 0.001$). This fact was reinforced by our study wherein MDA level was significantly high ($P < 0.001$) in diabetic patients.

Zadeh *et al*[6] compared the lipid peroxidation of 41 healthy and 87 NIDDM patients. The level of lipid peroxidation in NIDDM patient was 9.4 ± 3.3 micromol/l and this level was 4.1 ± 2.2 micromol/l in healthy subjects. This difference was highly significant statistically ($p < 0.005$).

Losada and Alio[7] estimated the serum concentration of MDA in 60 well-controlled type I diabetic patients (28 without retinopathy and 32 with retinopathy) and 13 age-matched healthy subjects. Patients with retinopathy showed significantly increased MDA level, 2.65 ± 1.00 micromol/l compared to diabetics without retinopathy 1.80 ± 0.81 micromol/l and healthy controls 1.47 ± 0.45.

Serum vit E levels were estimated to observe the antioxidant status of diabetics as well as normal healthy subjects. It was 7.97 mg/l (SD 0.62) and 11.48 mg/l (SD 0.35) in diabetic and control subjects respectively. The decrease in concentration of vit E level was highly significant statistically ($p < 0.001$).

Lower level of vit. E focuses indirectly towards increased oxidative stress in diabetic subjects in comparison to normal healthy subjects.

Serum vit E level was 7.43 mg/l (SD 0.13) in subjects who were suffering from diabetic complications whereas it was 8.62 mg/l (SD 0.25) in cases of diabetes without complications. The decreased levels of vit E in subjects with diabetic complication were statistically significant when compared with that of diabetic patients without complications ($p < 0.001$). Decrease levels of serum vit E in diabetics with complications in comparison to diabetic without complication favour further corroborative evidence of increase association of diabetic complications with higher oxidative stress.

In a similar study, Zadeh *et al*[6] selected 87 patients suffering from NIDDM and properly age and sex matched 41 healthy subjects.

The serum vit E level in NIDDM subjects was 19.6 micromole/l (SD 7.5) micromol/l and it was 23.8 micromol/l (SD 8.3) in healthy subjects. When compared, this difference of vit E concentration was statistically significant ($p < 0.05$). This observation favours our study.

Polidori *et al*[8] who have estimated vit E level in 72 type-2 diabetic patients and 75 normal healthy controls. The level of vit E was 18.6 (SD 1.2) micromol/l in diabetic patients and was 26.8 micromol/l (SD 1.0) in control subjects. When compared, this decrease of vit E in type-2 diabetic subjects was statistically highly significant ($p < 0.001$). Our observation also supports this fact.

Conclusion

It was concluded from the above observation that diabetic patients suffer from more oxidative stress. When compared, oxidative stress is still higher in diabetic patients with complications than patients without complications. Although other factors play an equally important role, if not more, in the pathogenesis of diabetic complications, oxidative stress plays a significant role in diabetes and its complications. This fact is to be kept in mind when planning strategies for prevention of complications of diabetes mellitus for better quality of life of diabetics.

Acknowledgement

We are thankful to Dr. Anup Roy, Medical Superintendent-cum-Vice Principal of Calcutta Medical College and Hospital, Kolkata for allowing us to carry-out the study. We also indebted to the patients, relatives, and staff for cooperating in the study.

References

1. Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991; 40: 405-12.
2. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care* 1996; 19: 257-67.
3. Garcia Soriano F, Virag L, Jagtap P *et al*. Diabetic endothelial dysfunction; the role of poly (ADP-ribose) polymerase activation. *Nat Med* 2001; 7: 108-103.

4 Du X, Matsumura T, Edelstein D *et al*. Inhibition of GAPDH activity by poly (ADP-ribose) polymerase activates three major pathways of hyperglycaemic damage in endothelial cells. *J Clin Invest* 2003; 112: 1049-57.

5 Bambolkar S, Sainani G. Evalution of oxidative stress in diabetes with or without vascular complication. *JAPI* 1995; 43: 10-4.

6 Zadeh N *et al*. Relationships between plasma measures of oxidative stress and control in NIDDM. *Diabetologia* 1997; 40: 647-53.

7 Losada M, Alio JL. Malondialdehyde serum concentration in type-1 diabetic with and without retinopathy. *Doc Ophthalmol* 1996-97; 93: 223-9.

8 Polidori MC *et al*. Plasma levels of lipophilic antioxidants in very old patients with type-2 diabetes. *Diabetes Metab Res Rev* 2000; 16: 15-9.

ORIGINAL ARTICLE

JIACM 2010; 11(1) : 26-30

Gamma-glutamyltransferase (GGT) – a Novel Marker of Endothelial Dysfunction?

R Sharma, S Sharma**, GG Kaushik****

Abstract

***Background:** Research in the past few years has linked oxidative stress and inflammation to β-cell dysfunction resulting from chronic exposure to hyperglycaemia. Recent prospective studies have suggested that an elevated level of C-reactive protein and Gamma-glutamyltranferase enzyme is associated with subsequent development of diabetes.*

***Aim of the study:** The aim of the study was to examine the relationship between GGT and the marker of inflammation, i.e., C-reactive protein, in diabetic subjects.*

***Methodology:** The study was conducted on 150 subjects. Out of these, 50 were healthy controls and 100 were type 2 diabetics. Plasma glucose (fasting and post-prandial), serum high sensitivity C-reactive protein, glycosylated haemoglobin and serum Gamma-glutamyltranferase hepatic enzyme levels were measured.*

***Results:** Mean high sensitivity-C-reactive protein and Gamma-glutamyltranferase levels in type 2 diabetic subjects were significantly higher than the values in controls ($p < 0.001$). Further, a significant positive correlation was observed between Gamma-glutamyltranferase and high sensitivity-C-reactive protein in subjects with type 2 diabetes ($r = 0.312$, $p = 0.001$).*

***Conclusion:** The rise in levels of high sensitivity-C-reactive protein and Gamma-glutamyltranferase in diabetic subjects and their significant association might be a result of inflammation and oxidative stress in diabetes mellitus.*

***Keywords:** Type 2 diabetes, oxidative stress, inflammation, Gamma-glutamyltranferase, C-reactive protein.*

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterised by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both[1]. Type 2 DM is caused by a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. This form of DM, accounts for approximately 90 – 95% of those with DM and was previously referred to as non-insulin dependent diabetes mellitus (NIDDM), or adult-onset DM[1].

Research in the past few years has linked oxidative stress (OS) and inflammation to β-cell dysfunction[2,3] resulting from chronic exposure to hyperglycaemia, free fatty acid, or a combination of the two. A growing body of data[4] reinforces the concept that inflammation also plays an important role in the pathogenesis of type 2 DM and links DM with concomitant conditions with inflammatory components.

C-reactive protein (CRP) is considered to be a major inflammatory cytokine that functions as a nonspecific defence mechanism in response to tissue injury or infection. Recent prospective studies have suggested that an elevated level of CRP is associated with an increased risk of developing type 2 DM[5,6,7].

Prospective studies have described that high level of Gamma-glutamyltranferase (GGT) enzyme is associated with subsequent development of diabetes[8,9,10]. Recently, serum GGT has been recognised as a marker of OS[11]. Indeed, oxidative processes are key components of chronic inflammation, acting on multiple pathways and amplifying inflammatory reactions. Further, activation of inflammatory processes may contribute to the development of type 2 DM[8,9,10].

Since, oxidative stress appears to be a key component of many reactions associated with chronic inflammation, we found it interesting to study the levels of GGT (a liver enzyme), and CRP (an inflammatory marker) in diabetic subjects. Therefore, the aim of the study is to examine the relationship between GGT and the marker of inflammation, i.e., CRP, in diabetic subjects.

** Ph.D Scholar, ** Assistant Professor, *** Professor and Head, Department of Biochemistry, Jawaharlal Nehru Medical College, Ajmer – 305 001, Rajasthan.*

Material and methods

The study was conducted from 2006 - 2007 at Jawaharlal Nehru Medical College and associated group of Hospitals, Ajmer (Rajasthan). A total of one hundred and fifty subjects were enrolled for this study. Out of these, fifty were healthy controls (twenty-five males and twenty-five females with mean age 53.8 ± 8.63 years) and a hundred were type 2 diabetics (fifty males and fifty females with mean age 51.1 ± 10.1 years). The mean duration of diabetes in type 2 diabetic subjects was 10.6 ± 2.78 years respectively.

The local ethics committee approved the study. Before participation, volunteers were fully informed of the nature and purpose of the study and written consent was obtained from each.

Inclusion criteria

- Type 2 DM was diagnosed on the basis of American Diabetes Association 2008 criteria (fasting plasma glucose ≥ 126.0 mg/dl after repeat testing).
- All subjects were non-alcoholics and non-smokers.
- The diabetic subjects were on no other medications other than oral anti-diabetic drugs.
- Among the baseline parameters, systolic and diastolic blood pressures (SBP/DBP) were measured three times and averaged.
- All subjects were non-hypertensive.

Exclusion criteria

Subjects with nutritional deficiency, oestrogen therapy or active inflammatory diseases were excluded from the study.

Anthropometric measurements

Height (without shoes) and weight were measured, and body mass index (BMI) was calculated as kilogram divided by square of height in meters.

Biochemical measurements

Fasting blood samples were drawn from all subjects and analysed. Plasma glucose (fasting and post-prandial) concentrations were measured by enzymatic glucose oxidase-peroxidase (GOD-POD)[12], serum high sensitivity C-reactive protein (Hs-CRP) levels were measured by immunoturbidimetric method[13], glycosylated haemoglobin (GHb) levels were analysed by cation exchange resin method[14], and serum GGT hepatic enzyme levels were measured by modified kinetic colorimetric method[15].

Statistical evaluation

Data were expressed as mean ± standard deviation (SD). The means were compared using students 't' test. Pearson's correlation analysis was used for correlation of parameters measured. Analysis was two-tailed and a p-value ≤ 0.05 was considered as statistically significant.

Results

Baseline characteristics of the type 2 diabetic subjects and controls are given in Table I. Baseline clinical characteristics, viz., BMI, age, and blood pressure (SBP/DBP) did not differ in type 2 diabetic subjects and controls ($p = 0.21$, $p = 0.11$, $p = 0.87/0.71$). Mean fasting plasma glucose, post-prandial glucose, and GHb levels of type 2 diabetic subjects were significantly higher than control subjects ($p < 0.0001$) (Table I).

Mean hs-CRP levels in type 2 diabetic subjects (2.42 ± 1.01 mg/l) were higher than the values in controls (1.15 ± 0.37 mg/l) and were found to be statistically significant ($p < 0.001$) (Table II).

A similar trend was observed in GGT values in type 2 diabetic subjects when compared with controls ($p < 0.001$) (Table II).

Further, a significant positive correlation was observed between GGT and hs-CRP in subjects with type 2 DM ($r = 0.312$, $p = 0.001$) (Table III). However, the association of GGT and hs-CRP was non-significant in controls ($r = 0.041$, $p = 0.78$) (Table III).

Table I: Baseline clinical characteristics of healthy controls and type 2 diabetic subjects.

	MI (Kg/m^2)	Age (Years)	SBP/DBP (mm Hg)	FPG (mg/dl)	PPG (mg/dl)	GHb (%)
Healthy controls (n = 50)	23.1 ± 3.99	53.8 ± 8.63	128/77.2	83.4 ± 13.3	104.0 ± 9.95	6.18 ± 0.31
Type 2 diabetics (n = 100)	24.0 ± 3.70	51.1 ± 10.1	129/76.8	159.0 ± 48.4	237.0 ± 69.5	10.2 ± 1.36
P value	0.21 (NS)	0.11 (NS)	0.87/0.71 (NS)	< 0.0001 (HS)	< 0.0001 (HS)	< 0.0001 (HS)
Male						
Healthy controls (n = 25)	24.4 ± 3.7	54.4 ± 8.85	128/76.9	84.1 ± 13.4	105.0 ± 11.9	6.13 ± 0.30
Type 2 diabetics (n = 50)	23.4 ± 3.5	50.2 ± 10.3	129/76.5	151.0 ± 44.0	232.0 ± 72.6	10.1 ± 1.39
P value	0.39 (NS)	0.08 (NS)	0.78/0.81 (NS)	< 0.0001 (HS)	< 0.0001 (HS)	< 0.0001 (HS)
Female						
Healthy controls (n = 25)	23.2 ± 3.6	53.1 ± 9.78	128/77.4	82.6 ± 13.5	102.0 ± 7.65	6.23 ± 0.33
Type 2 diabetics (n = 50)	23.5 ± 3.7	52.1 ± 9.88	128/77.1	167.0 ± 51.6	242.0 ± 66.7	10.3 ± 1.33
P value	0.36 (NS)	0.64 (NS)	0.95/0.84 (NS)	< 0.0001 (HS)	< 0.0001 (HS)	< 0.0001 (HS)
Female versus male						
Healthy controls P value	0.39 (NS)	0.62 (NS)	1.0/0.78 (NS)	0.62 (NS)	0.41 (NS)	0.25 (NS)
Type 2 diabetics P value	0.27 (NS)	0.34 (NS)	0.68/0.64 (NS)	0.10 (NS)	0.50 (NS)	0.34 (NS)

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FPG: Fasting blood glucose; PPG: Post-prandial glucose; NS: Non significant; HS: Highly significant. Data expressed as mean value of the parameter assessed ± standard deviation.

Table II: Values of high sensitivity C-reactive protein (Hs-CRP) and Gamma-glutamyltransferase (GGT) in subjects studied.

Subjects	Hs-CRP (mg/l)	GGT (U/l)
Healthy controls	1.15±0.37	27.6±7.36
Males (n=25)	1.08±0.34	33.0±5.53
Females (n=25)	1.12±0.33	31.2±4.40
P value	0.68 (NS)	0.21 (NS)
Type 2 diabetics	2.42±1.01	35.4±8.90
Males (n=25)	2.32±0.99	32.8±9.21
Females (n=25)	2.45±1.02	37.9±7.86
P value	0.57 (NS)	0.003 (S)
Healthy controls versus Type 2 diabetics P value	<0.001 (HS)	<0.001 (HS)

NS: Non significant; HS: Highly Significant; S: Significant.

Data expressed as mean value of the parameter assessed ± standard deviation.

Table III: Pearson's correlation analysis between values of high sensitivity C-reactive protein (Hs-CRP) and Gamma-glutamyltransferase (GGT) in subjects studied.

Subjects	Parameters correlated	
Type 2 diabetic subjects	Hs-CRP	GGT
r value	0.312	
P value	0.001 (HS)	
Healthy controls		
r value	0.041	
P value	0.78 (NS)	

HS: Highly significant; NS: Non significant.

Values for BMI, age and SBP/DBP did not differ significantly in control males and females as well as diabetic males and females. However, males and females of type 2 diabetic group had significantly higher ($p < 0.0001$) (Table I) values of fasting glucose, post-prandial glucose and glycosylated haemoglobin compared to control group males and females.

Discussion

In the present study, a significantly high ($p < 0.001$) increase in serum GGT was observed in type 2 diabetics compared to healthy controls. The results were in accordance with many prospective studies where strong relationship between GGT concentration and incident of diabetes have been observed in non-alcoholics independently of classical cardiovascular risk factors[8,9,16]. The results of the study also indicate a significant increase in values of hs-CRP ($p < 0.001$) in diabetic subjects when compared to healthy controls.

Various risk factors are considered in the pathogenesis of diabetes and there is substantial evidence that oxidative stress (OS) is increased in diabetic patients and that glucose can substantially contribute to the increased production of reactive oxygen species (ROS)[11]. Beta cells of pancreas, and vascular endothelium, possibly have the lowest potential for scavenging oxygen free radicals .This suggests susceptibility of beta cells to oxidative stress[17,18].

It has been speculated that elevated GGT levels might be a defensive response to oxidative stress or, otherwise a marker of OS, being involved directly in the generation of ROS, especially in the presence of iron or other transition metals, inducing lipid peroxidation in human biological membranes[17,18].

Activation of inflammatory processes may contribute to the development of type 2 DM. Recent prospective studies have suggested that an elevated level of CRP is associated with an increased risk of developing type 2 DM[5,6,7].

Significant correlation between GGT and hs-CRP has been observed in the present study in diabetic subjects ($r = 0.312$, $p = 0.001$). Since oxidative stress processes might have an implication in chronic inflammation[11], it may be hypothesised that elevation in hs-CRP and the related oxidative stress would give rise to a subsequent inflammatory response.

In our study, we did not consider type 2 diabetic subjects with complications, and hence, did not assess GGT levels in these subjects; but, several population studies[19,20] have shown a strong cross-sectional association between serum GGT concentrations and many cardiovascular disease risk factors or components of insulin-resistance syndrome, including age, obesity, smoking, lack of exercise, blood pressure, dyslipidaemia, and diabetes mellitus. In addition, in prospective studies[21,22] baseline serum GGT concentration has been an independent risk factor for the development of cardiovascular or cerebrovascular diseases. Thus, we may put forward that oxidative stress may be the underlying cause of any of the complications of diabetes and it amplifies inflammatory processes too. Hence, assessment of levels of GGT and hs-CRP may in future studies serve as biomarkers for evaluating diabetic complications.

In conclusion, the rise in levels of hs-CRP and GGT in diabetic subjects and their significant association might be a result of oxidative stress resulting in diabetes. The follow-up of these diabetic subjects may further reflect findings of complications of DM.

The study proposes an association between GGT and hs-CRP which is essential. This may be considered as a limitation of the study. However, the study may be extended and carried-out on a large cohort to establish a causative relationship between GGT and hs-CRP.

As in the study only diabetic subjects without complications were considered, it may be considered as a limitation of the study. However, this study may prove important in future to assess the GGT and hs-CRP levels in type 2 diabetic subjects with complications and to evaluate the severity of complications (both micro and macrovascular). Thus, GGT and hs-CRP may be considered as sensitive biomarkers in predicting diabetes as discussed in the present study and in also evaluating the degree of complications in diabetic subjects in future studies.

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2008; 31 (Suppl. 1): S55-S60.
2. Ceriello A. Post-prandial hyperglycaemia and diabetes complications: is it time to treat? *Diabetes* 2005; 54 (1): 1-7.
3. Warram JH, Martin BC, Krolewski AS *et al*. Slow glucose removal rate and hyperinsulinaemia precede the development of type-II diabetes in the offspring of diabetic parents. *Ann Intern Med* 1990; 113: 909-15.
4. Ford ES. The metabolic syndrome and C-reactive protein, fibrinogen and leucocyte count: findings from the Third National Health and Nutrition Examination Survey. *Atherosclerosis* 2003; 168: 351-8.
5. Pradhan AD, Manson JE, Rifai N *et al*. C-reactive protein, interleukin-6 and risk of developing type 2 diabetes mellitus. *J Am Med Assoc* 2001; 286: 327-34.

6 Thorand B, Lowel H, Schneider A *et al*. C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: Results from the MONICA Augsburg Cohort Study, 1984-1998. *Arch Intern Med* 2003; 163: 93-9.

7 Laaksonen DE, Niskanen L, Nyyssonen K *et al*. C-reactive protein and the development of metabolic syndrome and diabetes in middle-aged men. *Diabetologia* 2004; 47: 1403-10.

8 Lee DH, Jacobs DR Jr, Gross M *et al*. Gamma-glutamyltransferase is a predictor of incident diabetes and hypertension: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Clin Chem* 2003; 49: 1358-66.

9 Nakanishi N, Nishina K, Li W *et al*. Serum gamma-glutamyltransferase and development of impaired fasting glucose or type 2 diabetes in middle-aged Japanese men. *J Intern Med* 2003; 254: 287-95.

10. Nakanishi N, Suzuki K, Tatara K. Serum gamma-glutamyltransferase and risk of metabolic syndrome and type 2 diabetes in middle-aged Japanese men. *Diabetes Care* 2004; 27: 1427-32.

11. Pleiner J, Mittermayer F, Schaller G *et al*. Inflammation-induced vasoconstrictor hyporeactivity is caused by oxidative stress. *J Am Coll Cardiol* 2003; 42: 1656-62.

12. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem* 1969; 6: 24.

13. Otsuji S, Shibata H, Umeda M. Turbidimetric immunoassay of serum C-reactive protein. *Clinical Chemistry* 1982; 28 (10): 2121-4.

14. Trivelli LA, Ranney HM, Lai HT. Haemoglobin components in patients with diabetes mellitus. *N Engl J Med* 1971; 284: 353-7.

15. Szasz G. A Kinetic Photometric Method for Serum Gamma Glutamyl Transferase. *Clin Chem* 1969; 15 (2): 124-36.

16. Lee DH, Ha MH, Kim JH *et al*. Gamma-glutamyltransferase and diabetes- a 4 year follow-up study. *Diabetologia* 2003; 46: 359-64.

17. Lim JS, Yang JH, Chun BY *et al*. Is serum gamma-glutamyltransferase inversely associated with serum antioxidants as a marker of oxidative stress? *Free Radic Biol Med* 2004; 37: 1018-23.

18. Drozdz R, Parmentier C, Hachad H *et al*. Gamma-glutamyltransferase dependent generation of reactive oxygen species from a glutathione/transferrin system. *Free Radic Biol Med* 1998; 25: 786-92.

19. Nystrom E, Bengtsson C, Lindstedt G *et al*. Serum gamma-glutamyltransferase in a Swedish female population. Age-related reference intervals; morbidity and prognosis in cases with raised catalytic concentration. *Acta Med Scand* 1988; 224: 79-84.

20. Nilssen O, Forde OH, Brenn T. The Tromso Study. Distribution and population determinants of gamma-glutamyltransferase. *Am J Epidemiol* 1990; 132: 318-26.

21. Wannamethee G, Ebrahim S, Shaper AG. Gamma-glutamyltransferase: determinants and association with mortality from ischaemic heart disease and all causes. *Am J Epidemiol* 1995; 142: 699-708.

22. Brenner H, Rothenbacher D, Arndt V *et al*. Distribution, determinants, and prognostic value of gamma-glutamyltransferase for all-cause mortality in a cohort of construction workers from southern Germany. *Prev Med* 1997; 26: 305-107.

FORM IV

The following particulars regarding the ownership of the **"JOURNAL, INDIAN ACADEMY OF CLINICAL MEDICINE"** are published as called for by Rule 8 of the Registration of Newspaper (Central) 1956.

1 Place of Publication – D-II/65, Kaka Nagar, Dr Zakir Hussain Marg, New Delhi – 110 003.

2 Periodicity of Publication – Quarterly

3 Printer's Name – B. B. Pevari
Nationality – Indian
Address – D-II/65, Kaka Nagar, Dr Zakir Hussain Marg, New Delhi – 110 003

4 Publisher's Name – B. B. Pevari
Nationality – Indian
Address – D-II/65, Kaka Nagar, Dr Zakir Hussain Marg, New Delhi – 110 003

5 Editor's Name – B. B. Pevari
Nationality – Indian
Address – D-II/65, Kaka Nagar, Dr Zakir Hussain Marg, New Delhi – 110 003

6 Name and address of individuals who own the newspaper and partners or shareholders holding more than one per cent of the total capital.

– Indian Association of Clinical Medicine, Headquarters: Postgraduate Department of Medicine, S. N. Medical College, Agra – 282 002 (U.P.)

I, Dr. B. B. Pevari, hereby declare that the particulars given above are true to the best of my knowledge and belief.

Sd/-
B. B. Pevari
Signature of Publisher

Date:
January 15, 2010

ORIGINAL ARTICLE

JIACM 2010; 11(1): 31-5

Aspiration Cytology for the Diagnosis of Tuberculous Lymphadenopathies: A Five-year Study

Rajesh Singh Laishram, RK Banashree Devi*, Ratan Konjengbam**, RK Tamphasana Devi***, L Durlav Chandra Sharma*****

Abstract

Background: Tuberculosis is still one of the biggest health challenges the world is facing. Tuberculous lymphadenopathy is an important form of extrapulmonary tuberculosis. The role of fine needle aspiration cytology (FNAC) in the diagnosis of such lesions is a well known fact.

Aims:

1. To study the distribution of cytomorphological patterns of tuberculous lymphadenitis.

2. To study the role of repeat aspiration in diagnosing tuberculous lymphadenitis.

Methods: The study was conducted in the department of pathology (cytology section) RIMS hospital for a period of 5 years from May 2002 to April 2007. Patients with peripheral lymphadenopathy having clinical suspicion of tuberculosis attending cytology OPD at RIMS hospital were enrolled for the study. Detailed clinical history regarding duration of swelling, sites, size, consistency, and mobility were taken into account. Fine needle aspirations were done and the smears were air-dried and stained with May-Grünwald Giemsa (MGG) and Ziehl-Neelsen (ZN) stain for AFB.

Results: Out of 4,024 aspirations from lymph nodes of various aetiologies, 1,210 cases were diagnosed as tuberculous (TB) lymphadenitis. Four cytomorphologic patterns were observed: 1) Caseating epithelioid granulomas: 616 cases (50.9%). 2) Granulomatous: 354 cases (29.25%). 3) Necrotising lymphadenitis: 161 cases (13.3%), and 4) Necrotising and suppurative: 79 cases (6.52%). AFB positivity was seen in 415 cases (34.29%). 361 cases were diagnosed as reactive lymphadenitis with activated histiocyte clusters and were advised a repeat aspiration after a course of antibiotics. Out of the 113 cases that turned-up for re-aspiration, 73 cases showed subsequent development of epithelioid granulomas, whereas the others still have reactive features.

Conclusion: FNAC is a useful tool in the study of tuberculous lymphadenitis and repeat aspiration, after 2 - 3 weeks helps in providing the correct diagnosis of early tubercular lesions.

Key words: Tuberculous lymphadenitis, caseating epithelioid granuloma, necrotising lymphadenitis.

Introduction

Despite progress in prophylaxis and therapy, tuberculous lymphadenitis still remains a rampant health problem in developing countries. With the arrival of HIV/AIDS in Manipur, tuberculosis (including tuberculous lymphadenitis) is on the rise. Fine needle aspiration technique was described first by Greig and Gray in 1904 who used this procedure in the diagnosis of trypanosomiasis. In 1921, Guthrie attempted to correlate lymph node aspiration cytology with various disease processes[1]. Since then there is no looking back. Various different cytomorphologies were encountered in day-to-day reporting of tuberculous lymphadenitis and typical epithelioid granulomas were found in some of the reaspirated cases diagnosed as reactive lymphadenitis with activated histiocytic clusters. Thus, this study was undertaken to determine the distribution of various cytomorphologies encountered, and the role of reaspiration cytology for demonstrating the subsequent development of granuloma.

Materials and methods

The study was conducted at the department of pathology, (cytology section), RIMS hospital for a period of 5 years, i.e., from May 2002 to April 2007. Patients with peripheral lymphadenopathy having clinical suspicion of TB and attending cytology OPD, RIMS hospital were enrolled for the study. Detailed clinical history including duration of swelling, sites, sizes, consistency, and mobility were taken into account before subjecting to aspiration.

** Demonstrator, ** Senior Demonstrator, *** Assistant Professor, *** Professor and Head, Department of Pathology, Regional Institute of Medical Sciences, Lamphel, Imphal - 795 004, Manipur.*

The relevant laboratory investigations, and chest X-ray were taken into account. After explaining the procedure to the patient, aspiration were done using 24G needle, 20 cc disposable syringe and Comeco syringe pistol. All the slides were air-dried and stained with MGG and ZN stain. Detailed cytomorphologies were studied. A repeat aspiration was advised in those patients diagnosed as reactive lymphadenitis with activated histiocyte clusters after 2 – 3 weeks.

Results

Out of the 4,024 aspirations from various peripheral lymph-adenopathies, 1,210 cases were diagnosed as TB lymphadenitis, and 361 cases as reactive lymphadenitis with activated histiocyte clusters. The age of the patient ranged from 6 months to 75 yrs with a median age of 34 yrs. The age group between 20 – 30 yrs was mostly affected (Table I). The male: female ratio was 1: 0.56. Among the various sites of lymph node involvements, cervical lymphnodes were the most common – 611 cases (50.49%) followed by axillary, inguinal nodes (Table II).

Table II: Table showing various sites of involvement.

S. No.	Sites	Number of cases (Total 1,210)	%
1	Cervical	611	50.49
2	Axillary	292	24.13
3	Inguinal	117	9.66
4	Supraclavicular	10	0.82
5	Multiple	180	14.87

A definitive cytologic diagnosis of TB lymphadenitis could be considered in the smears with the first two patterns, while the third and fourth could be dismissed as acute suppurative lymphadenitis in the absence of a positive ZN stain. Different patterns showed varied AFB positivity (Fig. 2) (Table III). The necrotising lymphadenitis and necrotising and suppurative lymphadenitis patterns

Tables I: Table showing various cytomorphological cases of TB lymphadenitis in relation to age.

S. No.	Age group (years)	Cytomorphologic patterns							
		Caseating Epithelioid granulomas (Total 616)		Granulomaous (Total 354)		Necrotising (Total 161)		Necrotising and suppurative (Total 79)	
		Number	%	Number	%	Number	%	Number	%
1	0 – 9	130	21.1	170	48.02	02	1.24	09	11.39
2	10 – 19	103	16.72	97	27.4	01	0.62	17	21.52
3	20 – 29	213	34.57	64	18.07	67	41.61	10	12.58
4	30 – 39	117	18.99	08	2.25	90	55.9	13	16.45
5	40 – 49	27	04.38	07	1.98	01	0.62	20	25.32
6	50 – 59	10	01.62	03	0.84	0		0	
7	60 – 69	14	02.27	02	0.56	0		0	
8	70 and above	02	0.32	03	0.84	0		0	

On the basis of cytomorphological analysis, TB lymphadenitis were categorised into 4 patterns (Table III):

1. Classical caseating epithelioid granulomas – 616 cases (50.9%) which showed epithelioid granuloma, caseation necrosis with or without giant cells in milieu of lymphoid cells (Fig. 1).
2. Granulomatous lymphadenitis – 345 cases (29.25%) which showed only epithelioid granuloma with or without giant cells.
3. Necrotising lymphadenitis – 161 cases (13.3%) which showed degenerating epithelioid cells in a necrotic background.
4. Necrotising and suppurative – 79 cases (6.52%) which showed degenerating and viable neutrophils in a necrotic background.

showed 100% AFB positivity. The other two patterns i.e., caseating epithelioid granuloma and granulomatous lymphadenitis had AFB positive only in 214 (34.75%) and 57 (16.02%) cases respectively. There were 282 (23.3%) cases of HIV positive cases in the study. 272/282 (80.49%) cases had the necrotising lymphadenitis (175 cases) and necrotising and suppurative patterns (97 cases).

Table III: Table showing cytomorphologic patterns with AFB positivity.

S.No.	Cytomorphology	Total/%	AFB positivity/%
1	Caseating epithelioid granuloma	616 (50.9)	214 (34.75)
2	Epithelioid granuloma	354 (29.25)	57 (16.02)
3	Necrotising	161 (13.3)	161 (100)
4	Necrotising and suppurative	79 (6.52)	79 (100)

Table IV: Comparison between various other studies.

S.No.	Study	Various cytomorphologies			
		Caseating EG	EG	Necro	Necro + Suppu
1	Ng *et al*[9]	18%	66%	7%	9%
2	Das *et al*[7]	39.1%	25.3%	35.6%	–
3	Llatjos *et al*[8]	30.43%	17.29%	52.17	–
4	Present study	50.9%	29.25%	13.3%	6.52%

EG: Epithelioid granuloma; Necro: Necrotising; Suppu: Suppurative.

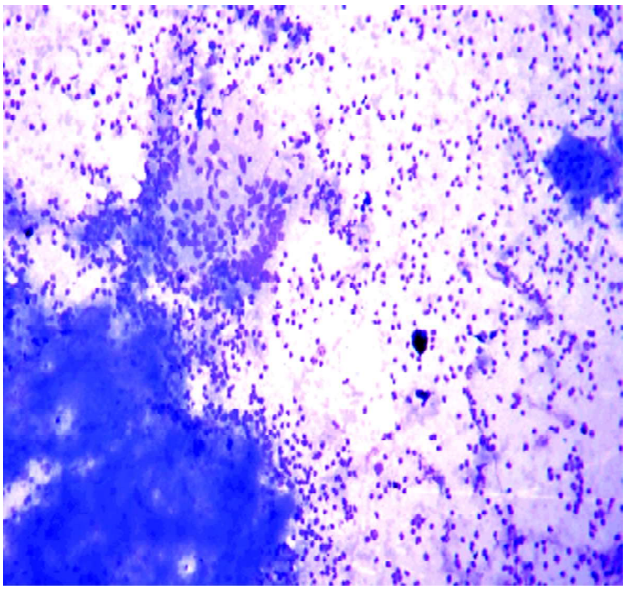


Fig. 1: *Microphotograph of FNAC lymph node smear showing caseating epithelioid granuloma (MGG stain, 10X).*

In addition to the above four groups, a fifth group comprising poorly developed/doubtful epithelioid cells or occasional epithelioid cells without characteristic necrosis/giant cells has been encountered. In this group, where there are micro foci of activated macrophages in a background of reactive lymphadenitis, a follow-up re-aspiration is required to look for subsequent development of granuloma. 361 cases were diagnosed as reactive lymphadenitis with activated histiocyte collection. These cases were advised a repeat aspiration after 2 - 3 weeks. 113 cases turned-up for repeat aspiration out of which 73 cases (64.61%) developed epithelioid granuloma, and AFB was seen in 30 cases. 40 cases (35.39%) still remained with reactive features (Fig. 3).

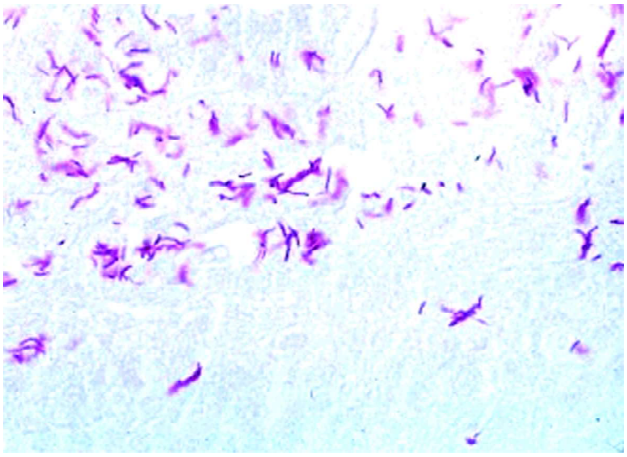


Fig. 2: *Microphotograph of FNAC lymph node smear showing AFB (ZN stain, 100X)..*

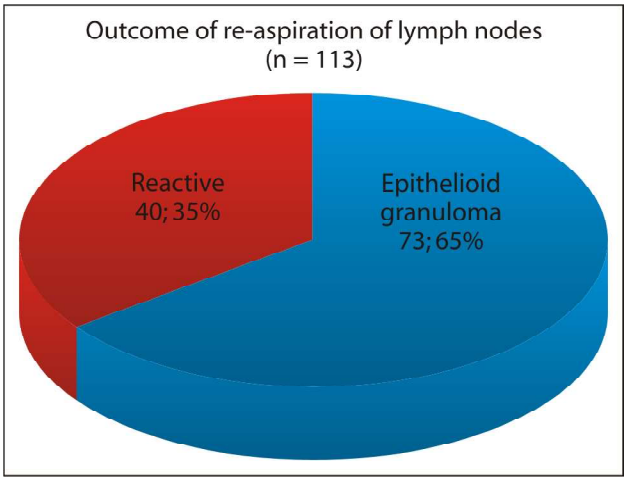


Fig. 1: *Pie chart showing the outcome of re-aspiration.*

Discussion

Tuberculosis being common in our country, it is not surprising that TB lymphadenopathy continues to be one of the commonest causes of chronic lymph node enlargement. Pandit *et al* stated that considering the overall prevalence of tuberculosis in the Indian context, the presence of epithelioid cell granulomas is indicative of tuberculosis[2]. Fine needle aspiration cytology in the diagnosis of tuberculous lymphadenitis is simple, safe, cost-effective, and conclusive[3]. The diagnostic cytomorphologic findings comprise epithelioid granulomas and giant cells – with or without necrosis[4]. Many-a-times an acute inflammatory exudate is obtained. AFB stain immensely augments diagnosis. Many workers have found many cytomorphologic patterns in the aspirates of tuberculous lymphadenitis. Metre and Jayaram[5] described three cytomorphologies: 1) Epithelioid granulomas with or without giant cells, 2) Degenerating epithelioid granulomas, and 3) Necrotising and suppurative. Similar to the study by Nayak *et al*[6], we found four patterns described. Das *et al*[7], and Llatjos *et al*[8], also described three patterns. Comparison of different cytomorphologic patterns by various workers is shown in Table IV. The cytomorphologic patterns to some extent denote the immune status of the individuals. Necrotising and suppurative patterns are more commonly seen in immunocompromised patients with a higher and heavy positivity of AFB. In a place like Manipur where HIV/AIDS has stormed, finding of such a pattern in a patient with unknown HIV status may reflect his immune status.

This study also showed that TB lymph node was not limited to younger age groups. No age group was exempted. Furthermore, the disease was not limited to the cervical lymph node either. Axillary and inguinal lymph nodes were also affected. These findings were similar to the study by Ng *et al*[9].

Detection of AFB in aspiration smears varies with the cytomorphological features in tuberculous lymph nodes. As was the observation by Malakar *et al*[10] we also found higher AFB positivity in smears containing necrotic materials.

TB lymphadenitis has unique stages (Jones *at al*[11]). Characteristically, more than than one lymph is involved in this disease. So, variable numbers of lymph nodes with variable stages are also a characteristic finding. These pathologic characteristics may be closely related to the outcome of fine needle aspiration cytology. Aspirates from stage one or two tuberculous lymphadenitis usually provide inflammatory cells as seen in reactive lymphadenitis. Thus, FNAC of these stages can only be nonspecific reactive. Typical necrotic materials or tubercle bacilli can be seen in the advanced stages in which an abscess is readily formed in the core of the lymph node[12]. So aspirates from an early stage lymph node were the main cause of low sensitivity. If lymph node aspiration was done once in the early stage, the diagnosis is likely to be dismissed as a reactive node. This study shows that re-aspirating after one or two weeks, i.e., waiting for the development of granuloma, the diagnostic efficacy improves. It is therefore necessary to follow the patients whose clinical findings are compatible with TB lesions.

Conclusion

FNAC being a reliable, safe, rapid, and economical procedure, is useful as an outdoor diagnostic procedure for the diagnosis of tubeculous lymphadenitis. From the cytomorphologic pattern and the AFB positivity, a rough estimate about the immune status can be made. The usefulness of repeat aspiration in patients with strong clinical suspicion of tuberculous lesions is well-documented in the study.

References

1. Narang RK, Pradhan S, Singh RP, Chaturvedi S. Place of fine needle aspiration cytology in the diagnosis of lymphadenopathy. *Ind J Tub* 1990; 37: 29–31.
2. Pandit AA, Khilani PH, Prayag A. Tuberculous lymphadenitis: extended cytomorphologic features. *Diagn Cytopathol* 1995; 12: 23–7.
3. Singh JP, Chaturvedi NK, Das A. Role of fine needle aspiration cytology in the diagnosis of tubercular lymphadenitis. *Indian J Pathol Microbiol* 1989; 32 (2): 101.
4. Verma K, Kapila K. Aspiration cytology for the diagnosis of tuberculosis-perspectives in India. *Indian J Pathol Microbiol* 2002; 69 (Suppl 1): S39–43.
5. Metre MS, Gita J. Acid fast bascilli in aspiration smear from tuberculous lymph nodes: An analysis of 255 cases. *Acta Cyto* 1987; 31: 17–9.

6 Nayak S, Puranik SC, Deshmukh SD *et al*. Fine needle aspiration cytology in the tuberculous lymphadenitis of patients with or without HIV infection. *Diagn Cytopathol* 2004; 31 (4) : 204-6.

7 Das DK, Pank JN, Chachra M *et al*. Tuberculous lymphadenitis: correlation of cellular components and necrosis in lymph node aspirate with AFB positivity and bacillary count. *Indian J Pathol Microbiol* 1990; 33: 1-10.

8 Llatjos M, Romeu J, Clotet B *et al*. A distinctive cytologic pattern for the diagnosing TB lymphadenitis in AIDS. *J Acquir Immune Defic Syndr* 1993; 6 (12) : 1335-8.

9 Ng WF, Kung ITM. Pathology of tuberculous lymphadenitis: A fine needle aspiration approach. *J Hong Kong Med Assoc* 1990; 42 (1) : 18-21.

10. Malakar D, Jajoo IIN, Kiran S *et al*. A clinical evaluation of fine needle aspiration cytology in the diagnosis of lymphadenopathy. *Ind J Tub* 1991; 38: 17-9.

11. Jones PG, Campbell PE. Tuberculous lymphadenitis in childhood: The significance of anonymous mycobacteria. *Br J Surg* 1962; 50: 302-14.

12. Suh KW, Park CS, Lee JT, Lee KG. Diagnosis of cervical tuberculous lymphadenitis with fine needle aspiration biopsy and cytologic examination under ultrasonographic guides. *Yonsei Med Journal* 1993; 34 (4) : 228-333.

REVIEW ARTICLE

JIACM 2010; 11(1): 36-9

Tuberculosis, Candidiasis, *Pneumocystis carinii* Pneumonia and Visceral Leishmaniasis Co-infections Associated with SIADH and Splenic Infarction in AIDS

SC Chaudhary, R Avasthi**, D Mohanty*, KP Singh***, U Rusia**, A Sharma*****

Abstract

Opportunistic infections (OIs) are the major cause of morbidity and mortality in patients with human immunodeficiency virus (HIV) infection. The most common opportunistic infection is tuberculosis, followed by candidiasis, infections causing diarrhoea, and Pneumocystis carinii pneumonia (PCP). We hereby report the case of a 36-year-old male with clinical stage IV acquired immunodeficiency syndrome (AIDS) who had multiple OIs including visceral leishmaniasis (VL) an uncommon co-infection in the Indian scenario. This patient also had features of syndrome of inappropriate secretion of anti-diuretic hormone (SIADH) and splenic infarction – a rare clinical problem in HIV.

Keywords: Opportunistic infection, Pneumocystis carinii pneumonia, visceral leishmaniasis, syndrome of inappropriate secretion of anti-diuretic hormone, splenic infarction, AIDS.

Introduction

HIV infection is a global pandemic. Cases have been reported from all states and union territories of India[1]. Though the overall prevalence of HIV infection is low (< 1%), the total number of cases is high. HIV seems to be affecting the economically productive, sexually active group; and majority of patients are migrant males, thus having a tremendous impact on the livelihood of the affected family[2]. OIs are the major cause of morbidity and mortality in patients with HIV infection. The spectrum of OIs depends upon the CD4 cell count and a highly significant inverse correlation has been observed. We hereby report the case of a 36-year-old male who was initially diagnosed as a case of VL and found to be HIV positive, had multiple OIs associated with SIADH and splenic infarction.

Case report

A 36-year-old male resident of Bihar presented with high grade fever associated with chills and rigors, anorexia, weight loss, and heaviness in the left upper abdomen of 5 – 6 months duration. There was no history of petechiae or purpurae, vomiting, loose motions, cough, haemoptysis, dyspnoea, tuberculosis in the past and the patient also denied any history of contact with tuberculosis. On examination, he was ill looking, had a uniformly dark pigmentation all over the body. However, mucosal pigmentation was absent. Signs of dehydration, sternal tenderness, and lymphadenopathy were also absent. He was normotensive, had severe pallor and massive hepatosplenomegaly. Fundus examination showed anaemic retinopathy. The rest of the systemic examinations were unremarkable.

Investigations revealed haemoglobin of 5.5 g/dl, total leucocyte count 4,400/mm^3, differential leucocyte count $P_{66}L_{32}E_1M_1$, platelet count 1,71,000/mm^3, and erythrocyte sedimentation rate of 138 mm in 1st hour. Peripheral smear showed severe anaemia, microcytic type along with reticulocytosis and lymphocytosis. Blood sugar, blood urea, and serum creatinine were normal. Serum electrolytes (sodium/potassium) were 126/4.8 mEq/l. Total serum bilirubin was 0.6 mg/dl, serum alanine aminotransferase 38 U/l, serum aspartate aminotransferase 40 U/l, serum alkaline phosphatase 429 U/l (33 – 96 U/l), and total serum protein was 10.8 g/dl (albumin 2.1 g/dl). Prothrombin time was 17 sec (control = 13 sec), and activated partial thromboplastin time was 42 sec (control = 28 sec). Urine examination revealed albuminuria (+++), and 24 hrs urinary protein was 1,108 mg. HBsAg and anti-HCV were negative. X-ray chest was normal. Ultrasound abdomen showed hepatosplenomegaly with multiple abdominal

** Ex-Senior Resident, ** Professor, *** Senior Medical Specialist, **** Postgraduate Student,*
Departments of Medicine and Pathology, University College of Medical Sciences (University of Delhi), and GTB Hospital, Shahdara, Delhi – 110 095.

lymphadenopathy. Indirect fluorescent antibody test for kala azar was positive (1:400) and bone marrow smear examination revealed *Leishmania donovani* (LD) bodies (Fig. 1). A diagnosis of VL was made and patient was put on tablet Miltefosine 50 mg BD for 4 weeks. After completion of treatment he had no fever, the size of his liver and spleen regressed; but he continued to have anaemia (haemoglobin 5 g/dl), raised serum alkaline phosphatase 974 U/l (33 – 96 U/l), total protein 8 g/dl (albumin 2 g/dl), persistent hyponatraemia (serum sodium/potassium – 125/3.7 mEq/l). Repeat bone marrow smear became negative for LD bodies. Repeat chest radiograph revealed mediastinal widening. Considering the above findings, contrast-enhanced computed tomography (CECT) abdomen and chest were done which revealed conglomerated, necrotic lymph node masses in mediastinum with right para-hilar infiltrates (Fig. 2), hepatomegaly, multiple periportal, peripancreatic retroperitoneal mesenteric lymph nodes, splenomegaly with splenic infarct (Fig. 3). CT-guided fine needle aspiration cytology from abdominal lymph node revealed caseating granuloma and was positive for acid-fast bacilli (AFB) stain. ELISA for HIV was positive, and CD4 cell count was 36/µl. There was no evidence of cytomegalovirus retinitis on fundus examination. Meanwhile he also developed dry cough, progressively increasing dyspnoea and swelling in both lower limbs. Sputum smear examinations were negative for Gram's and AFB stain, positive for *Pneumocystis carinii* and *Candida*. Sputum culture revealed *Candida albicans*. Repeated arterial blood gas analysis had shown severe hypoxaemia.

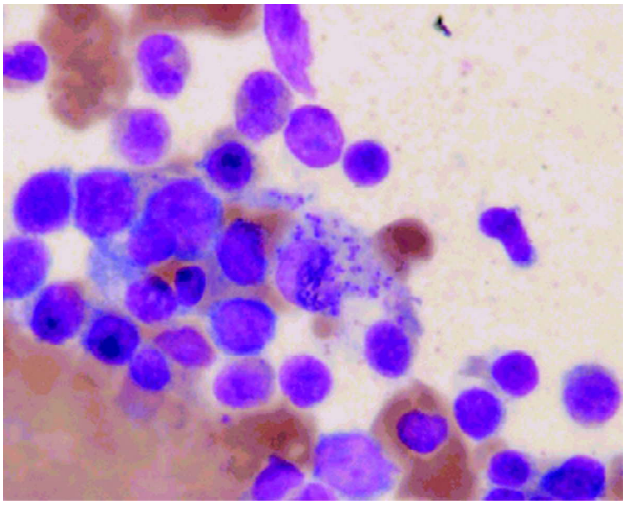


Fig. 1: *Bone marrow smear showing intracellular as well as extracellular LD bodies (Wright's stain 100 X).*

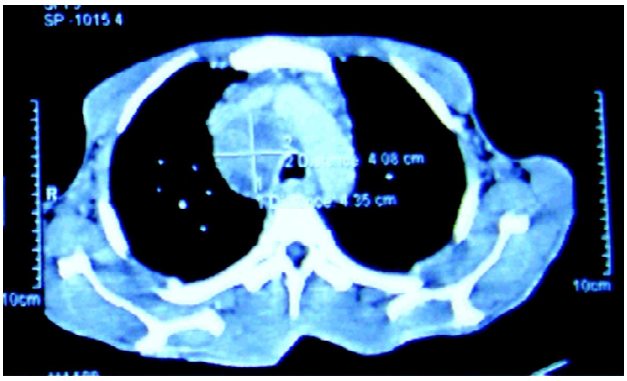


Fig. 2: *Contrast-enhanced computed tomography of chest showing conglomerated necrotic mediastinal lymph node mass.*

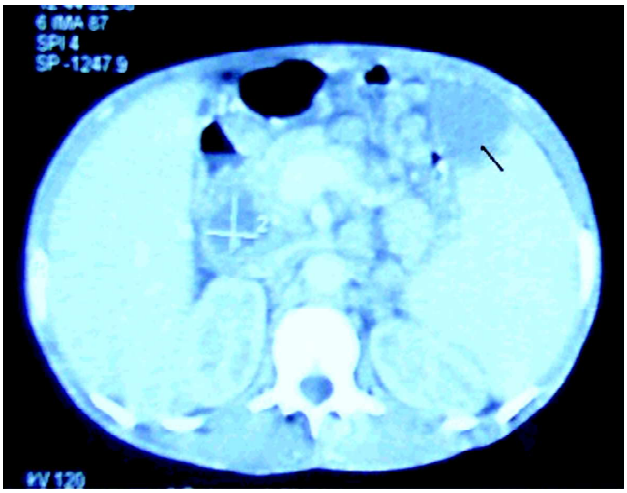


Fig. 3: *Contrast-enhanced computed tomography of abdomen showing conglomerated necrotic lymph node mass along with splenic infarction (arrow).*

In view of persistent hyponatraemia along with normal serum potassium in absence of dehydration/fluid overload and mucosal pigmentation in a normotensive patient, fasting serum cortisol and thyroid function test were done which came out to be normal. His urinary sodium and osmolality were 46 mmol/litre and 120 mOsm/kg of water respectively. A final diagnosis of clinical stage-IV AIDS associated with multiple OIs: tuberculosis, PCP, *Candida albicans* and VL associated with SIADH and splenic infarction was kept. He was started on trimethoprim/sulphamethoxazole (TMP/SMX), fluconazole, intravenous antibiotic, demeclocycline, anti-tubercular therapy (ATT) and extra oral salt in diet. Anti-

retroviral therapy (ART) was planned. The patient progressively deteriorated and subsequently expired within 10 days of the above treatment.

Discussion

OIs being the major cause of morbidity and mortality in patients with HIV infections, needs urgent attention for early diagnosis and treatment. Most common OI is tuberculosis, followed by candidiasis, and infections causing diarrhoea and PCP. However, VL is reported only in about 1.1 – 1.2% patients in case series from India[2]. The clinical manifestations of tuberculosis in HIV-infected patients are quite varied and generally show different patterns as a function of CD4+ T-cell count. In patients with lower CD4+ T-cell counts, disseminated disease is more common. In these patients the chest X-ray may reveal diffuse or lower lobe bilateral reticulonodular infiltrates consistent with miliary spread, pleural effusion, and hilar/mediastinal adenopathy. Infection may be present in bone, brain, meninges, gastrointestinal tract, lymph nodes, and viscera[3].

Mycobacterium avium complex (MAC) infection is a late complication of HIV infection, predominantly occurring in patients with CD4+ T-cell count of < 50/µl. The average CD4+ T-cell count at time of diagnosis is 10/µl. The most common presentation is disseminated disease with fever, weight loss, and night sweats. At least 85% of patients with MAC infection are mycobacteraemic, and large numbers of organisms can often be demonstrated on bone marrow biopsy. It has been suggested that prior infection with *M. tuberculosis* decreases the risk of MAC infection. Drug therapy consists of macrolides, usually clarithromycin, with ethambutol. The possibility of MAC infection was considered but not demonstrated in our case[3].

PCP, once the hallmark of AIDS, has dramatically declined in incidence following the development of effective prophylactic regimens and the widespread use of combination of ART. The risk of PCP is greatest among those who have CD4+ T-cell counts < 100/µl and 95% patients have CD4+ T-cell count < 200/µl. A definitive diagnosis of PCP requires demonstration of the trophozoite or cyst form of the organism in samples obtained from induced or spontaneous expectorated sputum, bronchoalveolar lavage, transbronchial biopsy, or open lung biopsy; and the preferred regimen for prophylaxis is TMP/SMX, one double strength tablet daily[3].

Although India contributes heavily to the global HIV and VL disease burdens, information about co-infection in this country is surprisingly sparse (1.5 – 6.3% as reported by Thakur *et al*, 2003, Sinha *et al*, 2003; and Mathur *et al*, 2006) as compared to southern Europe where 50 – 75% of adult cases of VL are HIV positive (Alvar *et al*, 1997; Lopez-Velez *et al*, 1998; Pintado *et al*, 2001)[4]. A majority of HIV/leishmania co-infected cases show classical features of VL; however, splenomegaly is less common and may be absent in immunocompromised patient. Diagnosis is quite difficult as only 40 – 50% of VL/HIV co-infected cases have a positive leishmanial serology. Anti-leishmanial antibodies in HIV positive patients are 50 times less than those in HIV-negative patients. Therefore, there may be many false negative tests. Direct examination of amastigote in the splenic or bone marrow aspiration has been the gold standard[5]; however the rk-39 strips have a sensitivity and specificity of 95 – 100% which is a most feasible method at the primary health centre (PHC) level in the current scenario. Oral miltefosine seems to be the most appropriate drug for VL at a dose of 2.5 mg/kg for 28 days because of ease of administration, less side-effects, and low relapse rate.

The SIADH is the most frequent cause of hyponatraemia. Although hyponatremia associated with volume depletion of the extracellular fluid also occurs commonly. Certain populations are at increased risk for hyponatraemia associated with the syndrome of inappropriate antidiuresis (SIAD), and its incidence rises with increasing age. Althogh the causes of SIAD are myriad, they can be categorised as related to malignant diseases, pulmonary diseases, and disorders of the central nervous system. In addition, a variety of drugs can stimulate the release of arginine vasopressin or potentiate its action. Hyponatraemia is the most common electrolyte abnormality encountered in HIV-infected patients either due to SIADH or adrenal insufficiency, which prolongs the hospital stay, and its presence carries a bad prognosis[6]. The only definitive treatment of SIADH is elimination of its underlying cause. Asymptomatic patients with chronic

hyponatraemia have a low-risk of serious neurological sequelae but a well-described risk of osmotic demyelination with rapid correction. Therefore, treatment is aimed at correcting the hyponatraemia very gradually. Fluid restriction is a cornerstone of therapy. Demeclocycline (300 to 600 mg twice daily) reduces urinary osmolality and increases serum sodium levels, but its effects can be variable. Alternatively vasopressin receptor antagonist might be used although clinical experience remains very limited[7].

Splenic infarction is a rare clinical problem commonly associated with haematological disorders, systemic embolisation in the setting of left atrial or ventricular mural thrombus as a result of acute myocardial infarction and rarely in HIV infection. Clinical spectrum varies from asymptomatic infarction, discovered incidentally to haemorrhagic shock. However, most common presenting symptom is left upper quadrant abdominal pain, and surgery is indicated only in presence of complications such as haemorrhage, rupture, abscess, or pseudocyst.

Conclusion

In the present scenario as India is contributing significantly to the global burden of HIV, high index of clinical suspicion is required for early diagnosis and prompt treatment of such cases to prevent uneventful outcome.

References

1 Sharma SK, Kadhiravan T, Banga A *et al*. Spectrum of clinical disease in a series of 135 hospitalised HIV-infected patients from north India. *BMC infectious Disease* 2004; 4: 52.

2 Chakravarty J, Mehta H, Parekh A *et al*. Study of clinico-epidemiological profile of HIV patients in Eastern India. *J Assoc Phys India* 2006; 54: 854-587.

3 Fauci AS, Lane HC. HIV disease: AIDS, related disorders. In: Kasper DL, Braunwald E, Fauci AS *et al*, eds. Harrison's Principles of Internal Medicine, 17th ed. McGraw-Hill Medical Publishing Division; 2008; 1137-1204.

4 Mathur P, Samantaray JC, Vajpayee M, Samanta P. Visceral leishmaniasis/human immunodeficiency virus co-infection in India: the focus of two epidemics. *J Med Microbiol* 2006; 55: 919-22.

5 Sinha PK, Pandey K, Bhattacharya SK. Diagnosis and management of leishmania/HIV co-infection. *Indian J Med Res* 2005; 121: 407-14.

6 Sridhar CB, Isaac BC. Hyponatraemia in HIV-infected patient. *J Assoc Phys India* 1998; 46: 980-83.

7 Ellison DH, Beri T. The syndrome of inappropriate antidiuresis. *N Engl J Med* 2007; 356: 2064-72.

ACKNOWLEDGEMENT

LIST OF *JIACM* REVIEWERS (2009)

- Ajay Wanchu (Chandigarh)
- A.K. Tripathy (Lucknow)
- A.K. Agarwal (New Delhi)
- Ajay Ajmani (New Delhi)
- Ajay Wanchu (Chandigarh)
- Alladi Mohan (Tirupati)
- Aman Sharma (Chandigarh)
- Anil Mahajan (Jammu)
- Anita Sharma (Dehradun)
- B. Venganna (Tirupati)
- B.O. Toyade (Nagpur)
- Bhawani Singh (New Delhi)
- E. Chandrasekaran (New Delhi)
- R.K. Garg (Lucknow)
- Gautam Alhuwalia (Ludhiana)
- Geeta Khwaja (New Delhi)
- H.K. Kar (New Delhi)
- Harpreet Singh (Rohtak)
- J.R. Sankaran (Chennai)
- Kumud Rai (New Delhi)
- M.U. Rabbani (Aligarh)
- M.P. Sharma (New Delhi)
- Manjari Tripathi (New Delhi)
- Naveen Garg (Lucknow)
- Nihal Thomas (Vellore)
- O.P. Kalra (New Delhi)
- Praveen Aggarwal (New Delhi)
- Pushpa Yadav (New Delhi)
- R.N. Gupta (New Delhi)
- R.S.K. Sinha (New Delhi)
- Rajat Kumar (Canada)
- Rajesh Khadgawat (New Delhi)
- Rajesh Upadhyay (New Delhi)
- Rohini Handa (New Delhi)
- S. Anuradha (New Delhi)
- S.C. Sharma (New Delhi)
- S.K. Sharma (New Delhi)
- S.K. Verma (Dehradun)
- Sashi Khare (New Delhi)
- Sham Sunder (New Delhi)
- Sushil Modi (New Delhi)
- Tamilarasu Kadhiravan (Tirupati)
- Tushar Roy (New Delhi)
- Usha K. Baweja (New Delhi)
- Vimlesh Purohit (New Delhi)
- Vineet Talwar (New Delhi)

REVIEW ARTICLE

JIACM 2010; 11(1): 40-5

Peripheral Vascular Disease – a Physician's Perspective

Mridul Chaturvedi, Shashank Dixit**, Lalrinamawia**, Rajeev Kumar***

Introduction

As India is now becoming a capital of diabetes all over the world. PVD (peripheral vascular disease or peripheral arterial disease) is a relatively lesser known entity in diabetic patients as compared to the other long-term complications. As the rising epidemic of diabetes is encroaching Indian population, incidence of PVD is also increasing and it is now often seen by a physician practicing at community level. In the present article, we will discuss some of the basic methods for the assessment of PVD, and its possible management at the community level.

Clinical approach to a case of PVD

A good history and physical examination is the cornerstone in the diagnosis of PVD. Detailed information regarding the age, gender, occupation, associated medical problems, and risk factors should be obtained. The history should focus on identifying the risk factors for atherosclerosis. The past medical history should concentrate on prior vascular events such as MI, stroke, amputation, DVT, or revascularisation in any vascular bed. History of smoking (current or reformed) should be elicited. The most characteristic features which are found in case of PVD are claudication, ischaemic rest pain, and skin ulceration, or dry gangrene[1].

Claudication (limping) is a reproducible discomfort/ache/pain in single or multiple muscle groups of the lower extremity brought in a predictable manner by exercise (walking). It is relieved with rest (cessation of walking), usually within 5 to 10 min[2]. Claudication needs to be differentiated from other causes of leg pain, mainly arthritis and sciatica. Unlike arthritis, claudication pain occurs in the muscles rather than in joints. In sciatica, the pain radiates down the back of the leg, is often associated with backache, may become worse on coughing or sneezing, and is not relieved by taking rest. The distance that a patient can walk before he is forced to take rest is known as 'claudication distance' or 'maximum walking distance' (MWD). This is a good indicator of severity of the disease; shorter the MWD, more severe is the disease.

Ischaemic rest pain is more worrisome. It refers to severe, continuous, relentless pain in the extremity (usually toes, or forefoot) due to grossly inadequate perfusion. Often worse at night (night pain), it is partially relieved by sitting or hanging the foot by the side of the bed. The pain is so severe that common analgesics fail to relieve it. It is said to be due to the cry of the dying nerves (due to severe ischaemia).

Skin ulceration and dry gangrene are late manifestations of disease, due to severe compromise in the blood supply. Ulcers which fail to heal within two weeks should raise the suspicion of PVD; they are often compounded by neuropathy and soft tissue infection.

Physical examination

The physical examination in patients with PVD should consist of careful inspection and palpation. Observations should note any asymmetry between the limbs, joint deformities, varicose veins, skin discoloration, and absence of hair over toes, swelling, ulcerations, tissue loss, and gangrene.

On inspection, skin may have atrophic, shiny appearance, and may demonstrate trophic changes, including alopecia, dry, scaly, or erythematous skin and brittle nails.

Advanced PVD may manifest as mottling, "fish-net pattern" (livedo reticularis).

- Palpation of the pulses (femoral, popliteal, dorsalis pedis, posterior tibial, carotid, brachial, and radial) is a mandatory part of the routine physical examination. Absence of pulses is the most specific sign of PVD. It is useful to remember that the dorsalis pedis artery may not be palpable in 10% of population.

** Assistant Professor, ** Postgraduate Student, Department of Medicine, S. N. Medical College, Agra – 282 002, U.P.*

Wide and prominent femoral and popliteal pulse may be a sign of aneurysm.

- A significant temperature difference between ipsilateral and contralateral limbs often is a sign of advanced disease.
- The carotid arteries, abdomen and femoral arteries should be auscultated for bruits.

Co-morbidities are common in PVD, and should be carefully assessed. These include the cardiac (dyspnoea, heart failure, angina, past history of CAD/PTCA/CABG, etc.), cerebral (TIA/stroke), renal (urea/creatinine), pulmonary (chronic cough, COPD), and diabetic (RBS, HbA1C) status.

Non-invasive testing

Ankle-brachial index (ABI)

Asymptomatic patients with peripheral vascular disease can easily be detected by the ankle-brachial pressure index which has a sensitivity of 97% and specificity of 100% in diagnosing PVD.

It is defined as the highest systolic blood pressure (SBP) of either the dorsalis pedis artery or posterior tibial artery divided by the higher of SBP from either the right or left brachial artery. It requires the use of a pocket Doppler and sphygmomanometer cuff. The normal value of ABI is more than 1.0. A value less than 0.9 indicates presence of occlusive arterial disease (PVD). ABI correlates well with the severity of the disease: lower the ABI, more severe is the disease. ABI increases after exercise in a normal person. A fall in ABI on walking also points to PVD.

The following table demonstrates the severity of PVD as per ABI and its relation with exercise and claudication[1,2]:-

ABIs can be falsely elevated in diabetics and patients on chronic haemodialysis.

Doppler ultrasound

The most widely used continuous wave (CW) doppler devices are simple bed-side, hand-held units that are economical and are easily carried. Doppler detects blood motion and may be used alone as a means of screening for vascular disease[2].

In a normal artery the wave form is triphasic. During the cardiac systole, there is forward flow in arteries. During early diastole, the flow reverses direction (because of elastic recoil of peripheral arteries). Finally, during middle to late diastole, there is return of forward flow as arterial blood runs off through the distal vessels. If the degree of stenosis is minimal, subtle changes such as dampening of signal and/or loss of the mid-diastolic forward flow component may be noted distal to the lesion, resulting in a biphasic signal. As the degree of stenosis worsens, the signal eventually becomes monophonic as only systolic flow is present. This same doppler is used to calculate the ABI.

Color doppler ultrasound (duplex scan)

The blood vessels can be visualised, and the blood flow in them demonstrated by this non-invasive investigation. This is an excellent modality to diagnose PVD, and to demonstrate the nature and extent of the arterial block. It is simple, safe, easy to perform, non-invasive, and has good correlation with angiography. However, it is operator dependant. Potential pitfalls of color doppler include visualising the tibial vessels, which are usually small and deep in the calf, and visualising the highly calcified vessels which are acoustically shadowed by calcium.

	Pre-exercise	Post-exercise	Claudication	Walking time (min)
Normal	> 1	> 1	None	5
Minimal	> 0.9	< 0.95	None	5
Mild	> 0.80	> 0.50	Present late	5
Moderate	< 0.80	< 0.50	Present limiting	< 5
Severe	< 0.50	< 0.15	Early limiting	< 3

When the arterial pressure is below 60 mmHg or ABI is below 0.5, pedal pulses are not palpable.

CT angiography/MR angiography

These two modalities have revolutionised the non-invasive assessment of PVD. They can be done on an outpatient basis, and the resolution of modern machines is excellent, and there is excellent correlation with digital subtraction angiography (DSA), which is an 'invasive' investigation. There is little to choose between CTA and MRA; MR has the advantage that it can be done in patients with compromised renal function. Angiography tells us (1) the site and extent of the block, (2) the state of arteries proximal to the lesion, and (3) the distal runoff (state of arteries beyond the block. These tests are mandatory prior to planning surgical revascularisation/ angioplasty. DSA can be done if concomitant evaluation of coronary arteries (coronary angiography) is indicated in a given patient.

Assessment of severity of disease

At the end of physical and non-invasive examination, one should be able to decide regarding the severity of disease. The Fontaine classification is extremely useful. The beauty of this classification is that it is based on clinical findings, and does not depend on any investigation.

Fontaine classification for severity of PVD

Stage I : Asymptomatic

Stage II : Intermittent claudication

(a) Non-disabling (mild)

(b) Disabling (severe)

Stage III : Rest pain

Stage IV : Tissue loss (non-healing ulcers/gangrene)

There can be some ambiguity about stage II and II(b). A 70-year-old retired man with a MWD of 200 meters (enabling him to shop, or to walk to the nearest bus-stand) would be labelled as II(a). However, the same MWD of 200 meters in a 50-year-old postman or courier-company worker would be labelled as II(b) (disabling, since this interferes in earning his livelihood). In general, a MWD of less than 100 – 150 meters qualifies as disabling (severe) claudication – stage II(b).

Management

The vast majority of PVD patients can be treated with medical therapy. However, if patient has tissue loss (ulceration/gangrene), or rest pain (Fontaine stages III and IV), these patients are labelled as 'critical ischaemia' and require prompt evaluation by a vascular surgeon for early intervention (surgery/angioplasty) because this is the only chance to save them for a major amputation. Medical therapy alone is rarely of help in critical ischaemia.

The co-morbid conditions (diabetes, hypertension, heart disease) should also be managed adequately. It is important to remember that 60 – 70% of patients with atherosclerotic PVD suffer from coronary artery disease, and 20 – 30% with cerebro-vascular disease – the two conditions which often kill these patients.

Medical management

Medical management consists of risk factor modification, which is as important as pharmacological management in a case of PVD.

- **Risk factor modification**
  - Patients should be encouraged and supported to stop smoking. Nicotine replacement products and bupropion are useful aids for tobacco cessation. This is perhaps the most important part of medical therapy.
  - Patient should participate in regular aerobic exercise/walking for at least 30 mins a day and at least 3 – 4 days in a week. They should be reassured that claudication is relatively benign, and their condition will not worsen if they walk to the limit of claudication (it will actually improve).
  - Patients should receive diet counselling and be encouraged to achieve the ideal body weight.
  - Diabetic patients should undergo yearly foot examination and should keep their blood sugar level well under control (HbA1c level < 7).
  - Hypertension should be controlled well. ACE-inhibitors have been shown to improve outcome

in patients with PVD. Beta-blockers are no longer contra-indicated in PVD, and should be used if the cardiac condition necessitates their use.

- **Drugs for peripheral vascular disease**

Drugs for hyperlipidaemia[3]

Several studies have shown that lipid-lowering therapy reduces the progression of atherosclerosis, and also have a plaque-stabilising effect. It has been found that statins improve the walking distance in patients with PVD.

LDL-C should be leveled to < 70 mg/dl. Statins should be considered first line therapy in addition to diet and exercise. Ezetimibe may be added to statin therapy in the treatment of peripheral vascular disease with moderate or intensive lipid lowering trial. Atorvastatin (80 mg/d) increases pain-free walking distance by 60%. Also, patients treated with statins have superior leg-functioning as assessed by walking speed and distance compared with those not so treated.

Anti-platelet therapy

Platelet inhibitors particularly aspirin, reduce the risk of adverse cardiovascular events in patients with peripheral atherosclerosis. Clopidogrel, a drug that inhibits platelet aggregation via its effect on adenosine diphosphate dependent platelet fibrinogen binding, is also effective in reducing cardiovascular morbidity and mortality in patients with PVD.

Aspirin (50 – 325 mg/day) and clopidogrel (75 mg/day) can be used alone or together when high-risk of vascular events is suspected.

Ticlopidine 250 mg BD may be used as an alternative to clopidogrel, but the risk of agranulocytosis must be kept in mind[4].

Cilostazol

It is a quinoline derivative that inhibits phosphodiesterase III, thereby decreasing cyclic adenosine monophosphate degradation and increasing its concentration in platelets and blood vessels resulting in inhibition of platelet aggregation, and vasodilation. It has been reported to improve absolute claudication distance by 40 – 50% as compared with placebo. Two RCTs have demonstrated that the drug is much superior to pentoxyfilline in improving claudication distance. The recommended dose is 50 mg PO twice daily, increasing to 100 mg twice daily. It is available under the trade name Zilast, Stiloz, and Pletoz in India. It is contraindicated in patients with heart failure. Common adverse effects are abdominal pain, diarrhoea, agranulocytosis, hypotension, and decrease in HDL-C[5,6].

Pentoxifylline[8]

An analogue of theophylline and phosphodiesterase inhibitor, it has been shown to increase blood flow in ischaemic areas by reducing whole blood viscosity and by improving flexibility of RBCs. The rheological property of vasodilatation is said to be responsible for improving passage of blood through microcirculation. It is usually well tolerated at dosage 400 mg TDS. Common side-effects are nausea, vomiting, dyspepsia and bloating sensation[8]. Recent published evidence suggests that pentoxyfylline is no better than placebo in management of claudication.

Ethaverine

It is an oral peripheral vasodilator usually indicated for peripheral vascular insufficiency with arterial spasm. It produces smooth muscle relaxation by acting directly on smooth muscle cells. The usual dosage is 100 – 200 mg TDS. Common side-effects are abdominal pain, arrhythmias, flushing, and headache.

Prostacyclin

It is an orally active analogue of the endogenous prostaglandin prostacyclin (PGI2). It exerts a direct vasodilatory action and inhibits platelet aggregation by interfering with thromboxane A2 initiated signals. In the treatment of intermittent claudication, a dose of 40 mcg PO tid is used[9]. Side-effects are headache, flushing.

Prostaglandin E1 liposomal (PGE1)

Prostaglandin E1 is formulated into a liposome product known as liprostin. It exerts a local vasodilator action, and has to be given intravenously. It is used in management of critical ischaemia (Fontaine III and IV when revascularisation/angioplasty is not technically feasible.

Naftidrofuryl

It is a peripheral vasodilator which significantly improves functional capacity in patients with intermittent claudication. It is given in doses of 200 mg TDS shown to improve pain-free walking distance by 37% as compared to placebo[10].

Angiogenic growth factors

Trafermin

It is a recombinant form of Beta Fibroblast Growth Factor (BFGF). It has been shown to protect neurons from the damaging effects of stroke – including oxygen and glucose deprivation. It has been given as 8 hour or 24 hour infusion.

Nicotinic acid derivatives

Its use as adjunctive therapy in the treatment of peripheral vascular disease has seriously been questioned. Nicotinic acid is active as a vasodilatory agent, and is useful in vasospastic conditions. The recommended dosage is 100 – 150 mg PO, given 3 – 5 times per day[11, 12].

Indigenous drugs

Ginkgo Biloba[13]

It is herbal product or dietary supplement. It is promoted to improve the symptoms of PVD, for the improvement of pain-free walking in patients with PVD or intermittent claudication. In a regimen including physical therapy, it should be given in an oral dosage of 120 – 160 mg per day PO in 2 – 3 divided doses. The side-effects are anaphylactic shock, bleeding, seizures, and oedema[14].

Surgery (peripheral bypass/angioplasty)

Revascularisation of the ischaemic limb is indicated in the presence of rest-pain, ulceration, or localised gangrene (Fontaine stage III and IV). This can be achieved by surgery (peripheral bypass or endarterectomy), or by angioplasty (with or without stenting). These two facilities should not be viewed as competing or mutually exclusive, but as complementary forms of therapy. In general, angioplasty is reserved for larger arteries (aorta, iliac), and short (discrete) occlusions, whereas bypass works better for long occlusions and in femoral or distal disease. Well-performed bypasses have 5-year patency rates ranging from 80% (femoro-popliteal bypass) to 95% (aorto-bifemoral bypass), and are vital in saving legs from (avoidable) amputations.

Conclusion

PVD is a common condition in the elderly, afflicting about 10% of the general population. Atherosclerosis is the commonest aetiology; Buerger's disease (thromboangiitis obliterans) is rare, and is seen in younger population (under 40 years of age). For the physician practicing at a community level, early recognition of PVD is very crucial; this can be achieved by simple clinical examination and clinical devices like ABI and bedside peripheral doppler. The management primarily focuses on risk factor modification and use of drugs like statins, aspirin, and cilostazol. Other drugs like prostaglandin analogues naftidofuryl, and pentoxyfylline, are of less proven benefit. Many of the patients with PVD (stage III and IV) require surgical intervention (peripheral bypass/angioplasty) by a vascular surgeon. The use of angiogenic growth factors (stem cell therapy) are the future ray of hope, but have to cover a long distance before coming into regular use in clinical practice.

References

1. Lakel RE. Peripheral Vascular Disease. *Text book of family medicine.* Saunders, An Imprint of Elsevier 7th Edition 2007; 39: 769-80.
2. Wennberg PW, Rooke TW. Diagnosis and Management of Disease of the Peripheral Arteries and Veins. *Hurst's – The Heart* 10th edition 90: 2421-7.
3. Creager MA, Libby P. Peripheral Arterial Disease. *Braunwald Heart Diseases* 8th Edition 57: 1503-05.
4. Creager MA, Loscalzo J. *Harrison's Principle of Internal Medicine* 17th Edition 243: 1569-70.
5. Dawson DL, Cutler BS, Meissner MH *et al*. Cilostazol has beneficial effects in the treatment of intermittent claudication. *Circulation* 1998; 98: 678-86.
6. Kunishima T, Musha H, Eto F *et al*. A randomised trial of Aspirin versus cilostazol therapy after successful coronary stent implantation. *Clin Ther* 1997; 19: 1058-667.
7. Ward A, Clissold SP. Pentoxifylline: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy. *Drugs* 1987; 34: 50-97.
8. Trental (Pentoxifylline) package insert. Bridgewater, NJ: Aventis pharmaceuticals Inc; 2004 Apr.

9. Lievre M *et al*. *Circulation* 2000; 102: 426-31.
10. Cochrane Database of Systemic Reviews 2008; 2 (Research report published online).
11. McKenney JM *et al*. A comparison of the efficacy and toxic effects of sustained versus immediate release niacin in hypercholesterolemic patients. *JAMA* 1994; 271: 672-7.
12. Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. *JAMA* 2001; 285: 2486-97.
13. Kleijnen J, Knipschild P. Ginkgo Biloba. *Lancet* 1992; 340: 1136-9.
14. Blumenthal M, Busse WR, Goldberg A *et al*. Ginkgo Biloba Leaf Extract. In: The Complete Commission E Monographs. *Therapeutic Guide to Herbal Medicines*. American Botanical Council, Austin, Tx 1998; 136-8.

Olmezest

CASE REPORT

JIACM 2010; 11(1): 46-8

Recurrent Diabetic Ketosis in Type-2 Diabetes: An Enigma

VP Singh, Vineet Jain**, Manjeet Singh**, AK Agarwal****

Abstract

Diabetic ketosis is a common medical emergency encountered by physicians. Early institution of appropriate treatment and comprehensive search for the cause of ketosis is of paramount importance. We present a patient with diabetes who was admitted to our hospital on five occasions in a short span of three months with a diagnosis of recurrent diabetic ketosis. He was investigated extensively during each admission but no apparent cause of ketosis could be definitely ascertained. However, during the fifth admission, he was investigated afresh for the presence of any occult infection and was detected to have evidence of disseminated extra-pulmonary tuberculosis.

***Keywords:** Occult infection, ileo-caecal tuberculosis, diabetic ketosis.*

Introduction

Diabetic ketosis is a disorder characterised by the presence of ketone bodies in serum and urine. It is different from ketoacidosis as there is no evidence of acidosis. Presence of ketones in a diabetic patient could be multifactorial. Infections, myocardial infarction, cerebrovascular accident, starvation, chronic alcohol consumption, presence of counter-regulatory hormone disorder, and poor compliance towards the treatment are some of the important causes.

Case report

A 42-year-old non-alcoholic, type 2 diabetic man on human insulin and having good glycaemic control, was admitted with complaints of acute abdominal pain and vomiting since one day. There was no history of fever, diarrhoea, dysuria, cough, or chest pain. The patient was dehydrated, had oral temperature of 99° F, pulse rate of 100/min, BP - 116/88 mmHg. His systemic examination was normal. His random blood sugar was 356 mg% and urinary ketones were present. The patient was treated with IV fluids, insulin, and other symptomatic treatment. Further investigations – i.e., haemogram, liver functions, kidney functions, serum amylase, ECG, chest X-ray, arterial blood gases and ultrasonography of abdomen – were normal. Blood and urine cultures were sterile. Within forty-eight hours he became asymptomatic and was discharged on subcutaneous 50 units of 70/30 human biphasic insulin in two divided doses.

The patient was readmitted within 15 days with similar complaints. This time, urinary ketones were present without acidaemia. There was neither evidence of any apparent infection nor any clinical evidence of starvation, missing meals or insulin, or any endocrine disease. He was again treated for diabetic ketosis. Routine haemogram, biochemistry, cultures, chest X-ray, and ultrasound abdomen were normal. This patient was subsequently discharged on adequate dosage of subcutaneous human insulin.

Within a span of two months he was admitted twice for similar symptoms of abdominal pain and vomiting along with headache. In addition to the above tests he was subjected to upper GI endoscopy, a CECT head, and CSF examination. Endoscopy revealed antral gastritis for

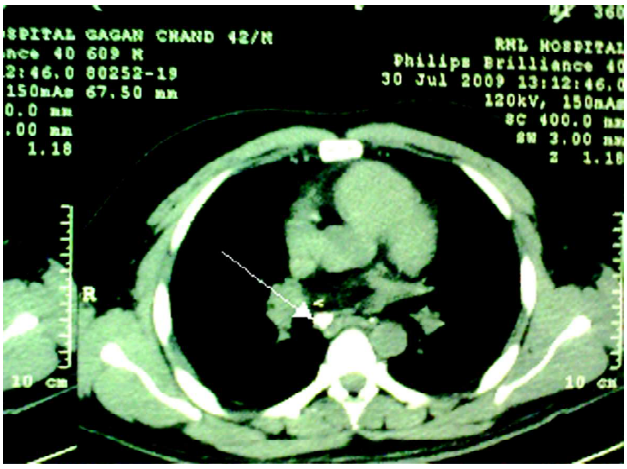


***Fig. 1:** A small calcified hilar lymph node (arrow).*

** Senior Resident, ** Post-graduate Student, *** Head of the Department of Medicine, and Dean, PGIMER, Dr. Ram Mahohar Lohia Hospital, Baba Kharak Singh Marg, New Delhi - 110 001.*

which *H. pylori* eradication treatment was prescribed. He responded well to this treatment and his abdominal symptoms subsided. CSF examination and CECT head were normal.

Surprisingly, the patient was admitted again for the fifth time with similar symptoms This time aggressive efforts were made to find an occult infection. Mantoux test, IgM ELISA for tuberculosis, HIV reactivity, blood, urine, and prostatic fluid cultures were all negative. A repeat USG abdomen, upper GI endoscopy and proctoscopy along with thyroid functions too were normal. Serum cortisol level was 8.17 µg/dl (normal levels: 4.3 – 22.4). He was subjected to a barium meal follow through (BMFT) which revealed thickening of caecum and pulling-up of the ileo-caecal junction suggestive of ileocaecal tuberculosis A small bowel barium enema also revealed a pulled-up ileo-caecal junction. Then a CECT of the abdomen and chest were performed on him, which showed few, small, calcified pre- and paratracheal, subcarinal, and right hilar lymph nodes with bilateral minimal pleural thickening and mild hepatomegaly. He was started on anti-tuberculosis therapy and continues to be on follow-up. He has not had any additional episodes of ketosis during this period.

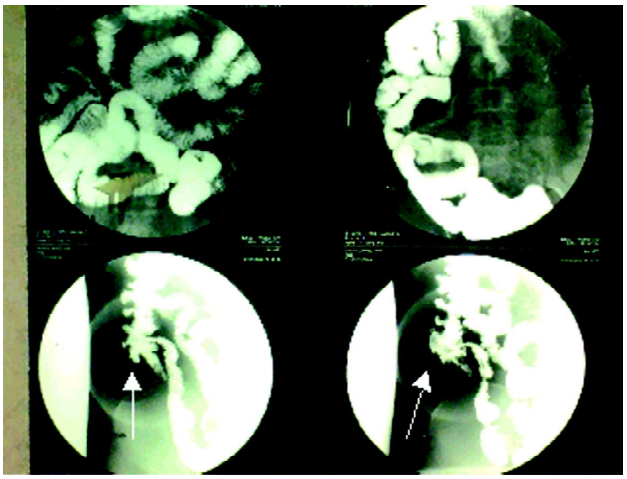


Fig. 2: *Barium meal with follow through showing pulled-up caecum (arrow).*

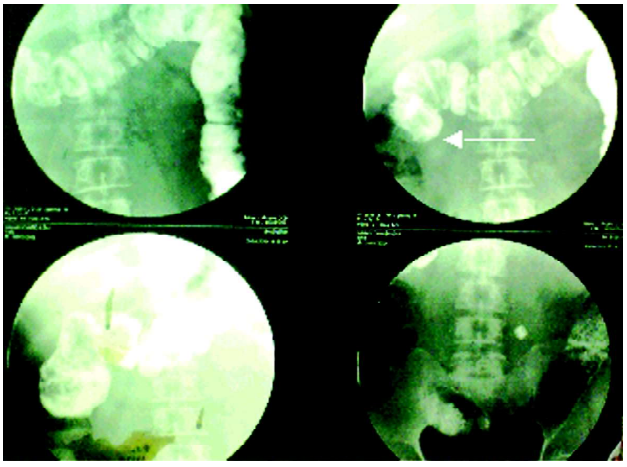


Fig. 3: *Barium enema showing shortening of ascending colon and pulled-up caecum (arrow).*

Discussion

Diabetic ketosis and ketoacidosis (DKA) are more common in type 1 diabetes. However, type 2 diabetics are also at risk during catabolic stress of acute infection, trauma, surgery and pregnancy. Two common precipitating factors in the development of DKA are inadequate or inappropriate insulin therapy and infection. Other precipitating factors include pancreatitis, myocardial infarction, cerebrovascular accidents, and drugs like corticosteroids, thiazide diuretics, and sympathomimetics like dobutamine and terbutaline[1].

The association between diabetes and increased susceptibility to infection is not supported by strong evidence[2]. Many infections are more common in diabetes *viz.* respiratory tract infection, urinary tract infection, soft tissue infection, and abdominal infections. Among non-pregnant adults with B group streptococcal bacteraemia, 27.5% were found to be diabetic[3]. A disproportionately high incidence (30 – 60%) of diabetes has been reported in patients with *Klebsiella* infections, liver abscesses and thyroid diseases.

Incidence of tuberculosis among patients with diabetes was found to be four times higher than the general population. Recently, the Asian community in England was found to have lung cavitation more commonly, particularly among diabetics[4]. Occult ileo-caecal tuberculosis, a paucibacillary disease, as a cause of ketosis remains a subject of controversy. Several aspects of immunity are altered in patients with diabetes. Polymorphonuclear function is depressed particularly when acidosis is present[5]. Our patient did not have acidosis during any of his admissions.

Occult infections such as oesophageal candidiasis, liver

abscess, splenic abscess, prostatitis, and pelvic inflammatory disease in females usually present with non-alarming symptoms such as difficulty or pain in swallowing, mild abdominal/pelvic pain, or per vaginal discharge. Hence, these conditions are often overlooked and under diagnosed.

Specific tests such as upper GI endoscopy, small bowel enema, and CECT abdomen can be used as tools for diagnosis of occult infections in cases of recurrent diabetic ketosis/ketoacidosis.

Various endocrinological abnormalities such as acromegaly and hyperthyroidism are also a cause of poor glycaemic control and cause recurrent ketosis/ketoacidosis in patients of diabetes. Four case reports have shown that patients with undiagnosed acromegaly may present with DKA as the primary manifestation of the disease[6]. Hence, in patients with recurrent diabetic ketosis/ketoacidosis with n2o overt or occult infection, associated endocrinological abnormalities should be suspected.

Infections remain the commonest cause of ketosis/ketoacidosis in patients of diabetes. Comprehensive and meticulous search for infections should be done for identifying the cause for ketosis/ketoacidosis.

References

1. Hyperglycaemic crisis in adult patients with diabetes. A consensus statement from the American Diabetes Association. *Diabetes Care* 2006; 29 (12): 2739-47.
2. Wheat LJ. Infection and diabetes mellitus. *Diabetes Care* 1980; 3: 187-97.
3. Farley MM, Harvey RC, Stull T *et al*. A population based assessment of invasive disease due to group B streptococcus in non-pregnant adults. *N Engl J Med* 1993; 328: 1807-11.
4. Hendy M, Stableforth D. The effect of established diabetes mellitus on the presence of infiltrative pulmonary tuberculosis in the immigrant Asian community of an inner city area of the United Kingdom. *Br J Dis Chest* 1983; 77: 87-90.
5. Joshi N, Caputo GM, Weitekamp MR, Karchnier AW. Infections in patients with diabetes mellitus. *N Engl J Med* 1999; 341: 1906-12.
6. Katz JR, Edwards R, Khan M, Conway GS. Acromegaly presenting with diabetic ketoacidosis. *Postgrad Med J* 1996; 72: 682-83.

CASE REPORT

JIACM 2010; 11(1) : 49-50

Salmonella typhi Endocarditis

VP Singh*, Vineet Jain**, Aarti Sharma**, AK Agarwal***

Abstract

Salmonella typhi is a common bowel pathogen, but is a rare cause of endocarditis. We report a case of endocarditis in a 24-year-old male who had clinically silent mitral valve disease and presented with prolonged pyrexia and diarrhoea. Bone marrow culture revealed presence of Salmonella typhi. He responded to ceftriaxone and gentamicin with disappearance of vegetations.

Key words: Salmonella typhi, rheumatic heart disease, salmonellosis, endocarditis.

Introduction

Cardiac involvement associated with *Salmonella* infection has been recognised for several years. Myocarditis occurs in 1 – 5% of cases while endocarditis is very rare[1]. Apparently 75% of cases have underlying cardiac abnormality, such as rheumatic heart disease or congenital heart disease. An apparent gastrointestinal or extra-intestinal focus is usually present in the course of the disease.

Case report

A 24-year-old male patient was admitted to the hospital with a history of high grade fever, diarrhoea, and prostration for two weeks. There was no history of cough, dyspnoea, dysuria, joint pains, or altered mental status. The patient had taken antibiotics for one week and remained afebrile for five days prior to this admission. He had no history of drug abuse, and did not use tobacco or alcohol.

On examination, the patient was febrile with a temperature of 103° F. He was dehydrated. His blood pressure was 100/70 mm Hg. He was mildly anaemic and non-icteric. No lymph nodes were palpable. Abdominal examination revealed mild hepato-splenomegaly. Cardiovascular and respiratory examinations were normal. Investigations revealed a haemoglobin of 9.5 gm%, TLC – 7,700 cells/ cumm, platelet count 1.1 lakh/cumm and ESR 98 mm in 1st hour, serum bilirubin was 2.3 mg%, ALT/AST were 88 and 70 respectively. Urine routine examination was normal. He was empirically treated with cefotaxime 3 gm/day (intravenous) and azithromycin 1 gm/day (intravenous). Mantoux test, HIV 1 and 2, HBsAg, anti-HCV, and malarial antigen test were negative. ECG and chest radiograph were normal. Ultrasound of the abdomen revealed mild hepato-splenomegaly. ANA and rheumatoid factor were negative. CRP was 2.8 mg%. Widal test was positive with a titre of 1:320 for *S. typhi* 'O' antigen. Stool, urine, and blood cultures were sterile. Bone marrow aspiration with culture was done. Patient remained febrile despite antibiotics. An HRCT chest and CECT abdomen to rule-out tuberculosis and a 2-D echocardiography to rule-out infective endocarditis as a cause of pyrexia were planned. HRCT chest and CECT abdomen were normal while 2-D echocardiography revealed presence of thickened anterior mitral valve leaflet, with vegetations attached to the anterior mitral leaflet cusp. Bone marrow culture grew *Salmonella typhi* sensitive to ceftriaxone, gentamicin, ampicillin, and ofloxacin. Hence the diagnosis of *Salmonella typhi* infective endocarditis was made on the basis of Duke's criteria. The patient was treated with ceftriaxone 2 gm IV/day and gentamicin 3 mg/kg IV/day for two weeks followed by ceftriaxone 2 gm IV/day for two more weeks. The patient recovered fully. A repeat 2-D echocardiography after two months revealed presence of only thickened anterior mitral leaflet with no evidence of vegetations – suggestive of complete recovery from endocarditis.

Discussion

Cardiac complications of salmonellosis are uncommon. Gram-negative organisms are implicated in 3 – 5% of native valve endocarditis, 3 – 13% in prosthetic valve endocarditis, and 5 – 13% in injection drug users, but

** Senior Resident, ** Post-graduate Student, *** Head of the Department of Medicine, and Dean, PGIMER, Dr. Ram Mahohar Lohia Hospital, Baba Kharak Singh Marg, New Delhi – 110 001.*

Salmonella endocarditis is rarely seen (1.3 - 4.8%)[2].

An apparent gastrointestinal or extraintestinal focus is usually present in the course of the disease. When cardiac involvement occurs there is predilection for involvement of previously diseased valve or endocardial surface. Frequently, more than one valve is simultaneously affected[3].

Salmonella serotypes commonly known to cause endocarditis include *S. choleraesuis, S. typhimurium, and S. enteritidis*. In addition, *Salmonella typhi* has been reported previously as a cause of endocarditis. Hewage *et al* reported the first case[4]. Tangia *et al* reported a case in a 24-year-old Egyptian woman, known to have rheumatic heart disease[5]. Du Plessis *et al* reported a case of right-side endocarditis with tricuspid regurgitation[6].

Non-typhoidal salmonellosis is also implicated in endovascular infections. The major risk factors for non-typhoid salmonellosis and bacteraemia are immunocompromised states, including extremes of age, alteration of endogenous bowel flora of intestine, diabetes, malignancy, autoimmune disorders, HIV infection, and therapeutic immunodeficiency[7]. The presence of primary bacteraemia (i.e., bacteraemia without associated gastrointestinal symptoms) should alert clinicians regarding associated immunodeficiency state.

The prognosis of *Salmonella* endocarditis is poor. It is often unsuspected and there is high incidence of acute valve destruction, thrombus formation with rapid cardiac decompensation. Infection of endocardium associated with MDR *Salmonella* carries poor prognosis.

The severity and site of involvement in salmonellosis are determined by virulence of the offending organism and presence of other debilitating diseases of the host. Ceftriaxone is the drug of choice for salmonellosis. Four-to-six weeks of antibiotic therapy, penicillin prophylaxis for rheumatic fever, and prophylaxis for endocarditis in special circumstances such as dental extraction, and surgical procedures is necessary.

The diagnosis of typhoid fever on clinical grounds is difficult, as the presenting symptoms are diverse and similar to those observed with other febrile illnesses. A definitive diagnosis can be made by isolation of *Salmonella typhi* from blood or bone marrow. Although the isolation of *Salmonella* from blood remains the method of choice for the laboratory diagnosis, blood cultures can be negative when patients have received prior antibiotic therapy[8].

Enteric fever is the only bacterial infection in humans for which bone marrow examination is routinely recommended. It is generally believed that bone marrow cultures will increase the diagnostic yield by about one-third compared with those from blood, despite the considerably lower volume of bone marrow required for culture. Effective antibiotic pretreatment had a significantly greater effect in reducing blood counts compared to bone marrow counts ($p < 0.001$). Thus, bacteria in the bone marrow of typhoid patients are less affected by antibiotic treatment than bacteria in the blood[9]. Bone marrow culture is the most sensitive test, being positive in nearly 90% of cases and can be used when a bacteriological diagnosis is crucially needed, or in patients who have been pretreated with antibiotics.

References

1. Kamili MA, Gazanfer A, Samia R *et al*. *Salmonella* infection mimicking acute rheumatic fever. *J Assoc Physicians India* 1996; 44: 579-80.
2. Khan GQ, Kadri SM, Hassan G *et al*. *Salmonella typhi* endocarditis: A case report. *J Clin Pathol* 2003; 56: 801-2.
3. Schneider PJ, Nernoff LTJ, Gold JA. Acute *Salmonella* endocarditis: Report of a case and review. *Arch Intern Med* 1967; 120: 478-82.
4. Hewage UC, Kamaladasa AI, Amarsinghe AK *et al*. *Salmonella typhi* endocarditis. *Ceylon Med J* 1994; 39: 43-4.
5. Tongia RK, Chowdhury MN. Endocarditis due to *Salmonella typhi*. *Trop Georg Med* 1983; 35: 187-8.
6. Du Plessis JP, Govendrageloo K, Levin SE. Right-sided endocarditis due to *Salmonella typhi*. *Pediatr Cardiol* 1997; 18: 443-4.
7. Ron-Bin Hsu, Yeou-Guang Tsay, Robert J Chen *et al*. Risk factors for primary bacteraemia and endovascular infection in patients without acquired immunodeficiency syndrome who have non-typhoid salmonellosis. *CID* 2003; 36: 829-34.
8. Malla T, Malla KK, Thapalial A *et al*. Enteric fever: A retrospective 6-year analysis of 82 paediatric cases in a teaching hospital. *Kathmandu University Medical Journal* 2007; 18: 181-7.
9. John Wain, Phan Van Be BaY, Ha Vinh *et al*. Quantitation of bacteria in bone marrow from patients with typhoid fever: Relationship between counts and clinical features. *J Clin Microbiol* 2001; 39: 1571-6.

CASE REPORT

JIACM 2010; 11(1) : 51-3

Bilateral Watershed Stroke following Cervical Injury – An Unusual Presentation

*Ashok Duggal**

Abstract

Watershed infarcts following cervical injury though a rare association can be suspected in a patient presenting in the the emergency room in altered sensorium along with features of cervical myelopathy.

Introduction

Cervical injury leading on to watershed infarcts in the brain is a rare association. Sudden acute cervical trauma in an apparently healthy individual may lead to vascular kinking at the cervical region resulting in hypoperfusion[1,2,3], thereby resulting in ischaemia/lacunar infarcts in the watershed zones producing a clinical picture of altered sensorium making the clinician to think about brain trauma with the principal presentation of cervical myelopathy, as happened in our case. This case is presented to enlighten the physicians regarding this rare clinical entity.

Case history

A 40-year-old man, painter by occupation, and in an apparently healthy state met with a roadside accident while travelling on a rickshaw (tricycle), and sustained neck injury but no head trauma. The patient developed deranged sensorium after about 45 minutes of the accident. Deranged sensorium lasted a few hours after the accident. Following the accident he developed retention of urine, and approximately six hours later progressive weakness of all the four limbs developed. Weakness started in the right upper limb, then involved the right lower limb, left lower limb, and left upper limb over the next 10 – 12 hours. Later on he developed difficulty in speech. The patient was advised MRI brain and cervical spine. Finally, MR angiography was planned but due to economic constraints could not be done.

Examination

On examination, the patient was in a state of confusion. His BP was 110/60 mmHg; pulse was 88/min; temperature was 98° F, and respiratory rate was 18/m regular. No cyanosis, jaundice, clubbing, or oedema was observed. There was no organomegaly or lymphadenopathy. Chest and CVS examination showed no abnormality. Examination of neck vessels revealed no bruit.There was no sign of meningeal irritation, and fundus exam was normal. Neck movements were restricted due to pain.

Next day, after recovery from confusional state, his speech revealed transcortical aphasia. Power was grade III in both upper limbs, and grade IV in both lower limbs. Deep tendon reflexes were exaggerated in right upper and both lower limbs, and ankle clonus was present in both lower limbs. Left upper limb reflexes were normal. Tone was increased as a whole, except in the left upper limb. Plantars were bilaterally extensor, and abdominal reflexes were absent. Sensations were impaired, but no level could be made out. The left hand initially had fasciculation, but after about two weeks developed atrophy of the small muscles.

Lab. investigations showed Hb – 13.8 gm%, TLC – 12,500/cumm, DLC – P_{80}, L_{16}, E_2, M_2, B_0. Platelet count – 2,75,000/cmm, RBS – 140 mg%, blood urea – 39 mg%, S. creatinine – 1.1 mg%, S. sodium – 140 mEq/l , S. potassium – 4.3 mEq/l, SGOT – 100 units/l, SGPT – 64 units/l; and PBF revealed normocytic normochromic picture. X-ray chest and abdominal ultrasound scan were normal. MRI 2D brain showed bilateral acute non-haemorrhagic infarction in watershed areas with old right MCA territory infarct.

MRI 3D cervical spine revealed left paracentral and foraminal disc protrusion with posterolateral osteophyte at C5-6 impinging upon the thecal sac, cord and the

* 2229, C – Block, Ranjeet Avenue, Amritsar, Punjab.

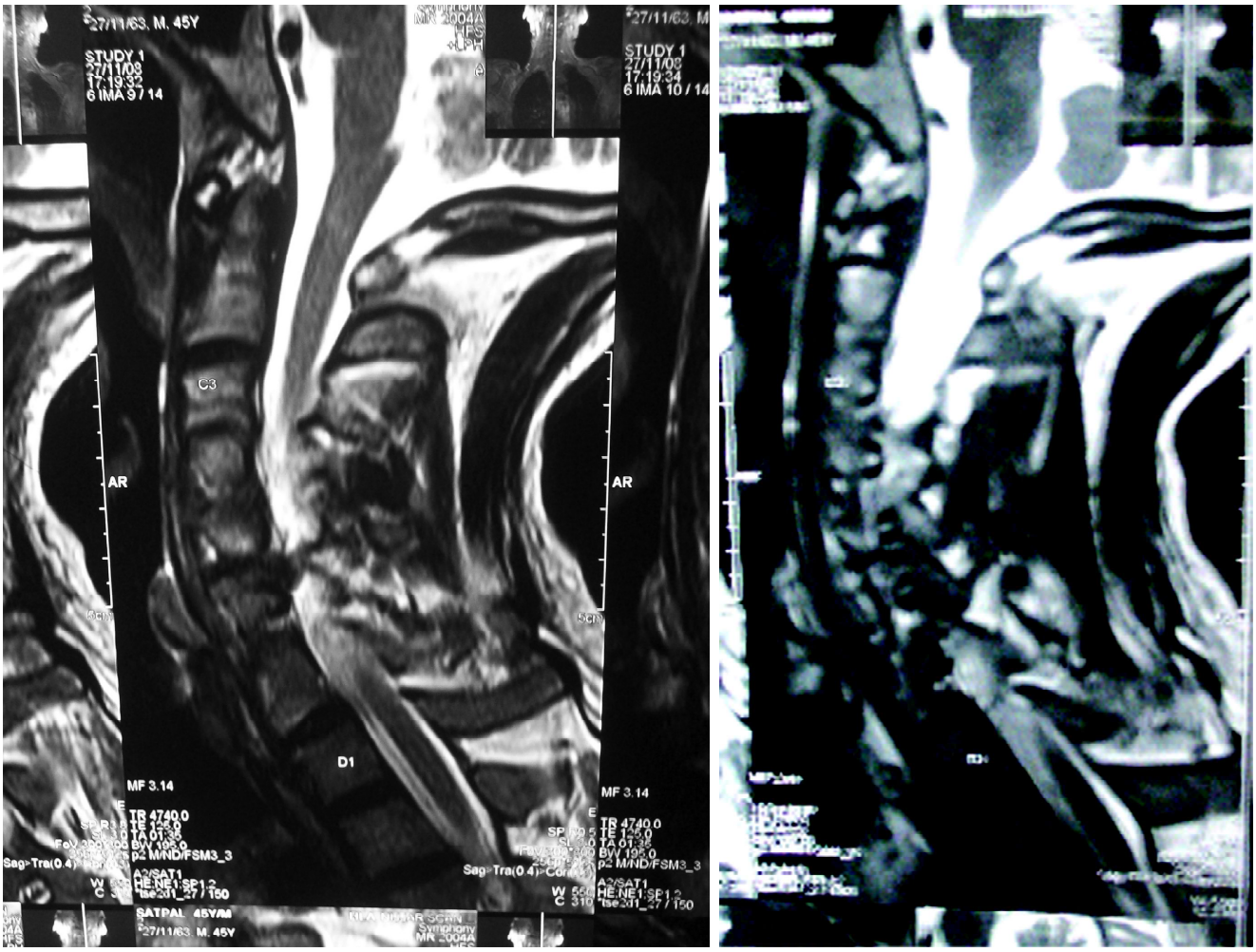


Fig. 1a and 1b: *T2 weighted Images of MRI cervical spine showing left paracentral and foraminal disc protrusion with posterolateral osteophyte at C5–6 impinging upon the thecal sac, cord, and the traversing left nerve root, with stenosis of left neural canal.*

traversing left nerve root with stenosis of left neural canal. Central focal bulge at C4–5 mildly indenting the thecal sac as well as diffuse disc bulge at C3–4 with mild thecal sac indentation was also observed.

Discussion

Watershed infarcts (boundary zone infarcts) by definition occur at the junction of distal territories of the two non-anastomosing arterial fields. Two areas have been described in literature. Cortical watershed (CWS) infarcts between the cortical territories of supply of ACA, MCA, and PCA. Internal watershed (IWS) infarcts occur in the deep white matter substance along and slightly above the lateral ventricle, between the deep and superficial arterial system of the MCA or between the superficial systems of MCA and ACA. Susceptibility of watershed zones is thought to result from their location at the distal field area where perfusion pressure is lowest. Pathophysiology of watershed infarcts remains debated. There may be two main situations which can result in hypotension, i.e., systemic hypotension (sudden and profound or repeated episodes of hypotension), and occlusion/extreme stenosis of carotid/vertebral arteries; but presence of both can complement each other. Occasionally, microemboli obstructing circulation (platelets, cholesterol, tumour) rather than low flow can cause infarction in the watershed zones. The occasional occurrence of syncope at the onset of watershed stroke, and later, the clinical picture of

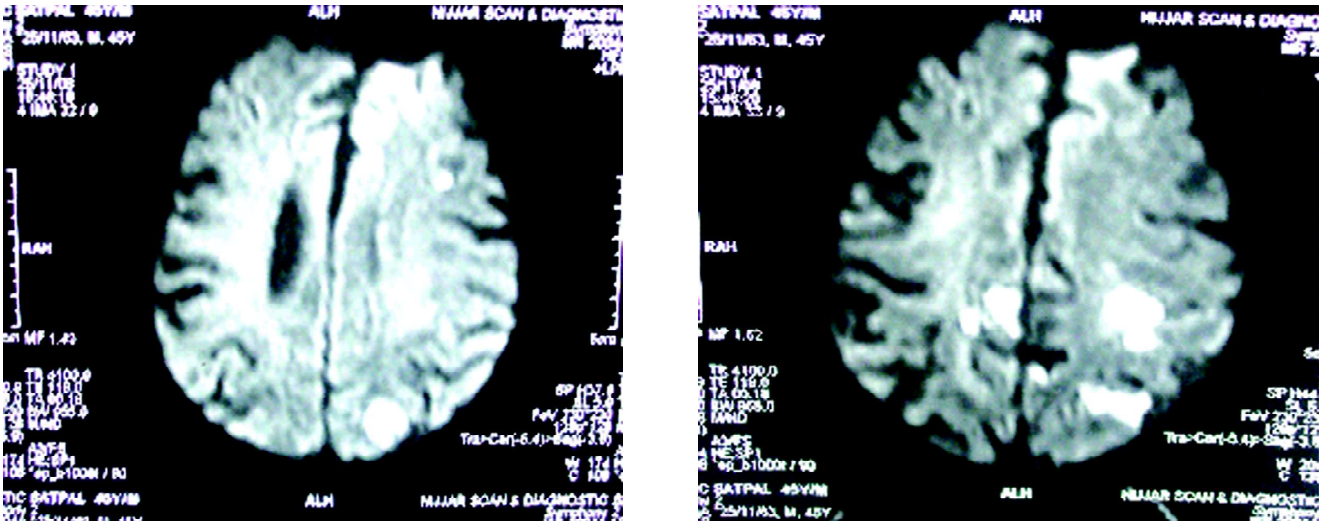


Fig. 2a and 2b: *T1 weighted images of MRI brain showing bilateral acute non-haemorrhagic infarction in watershed areas with old right MCA territory infarct.*

fluctuating or progressive weakness of the hand occasionally associated with upper limb shaking are consistent with haemodynamic failure. Anterior boundary zone and subcortical boundary zone infarcts are more common. This relative vulnerability may be because both MCA and ACA being the branches of same ICA. Unilateral infarcts or bilateral presentation will depend upon contralateral ICA disease and/or insufficient collateralisation. Conversely, stenosis or occlusion of vertebro-basilar system, or foetal type PCA, may favour the involvement of posterior watershed zone.

In our case, the patient had bilateral watershed infarcts which led to deranged sensorium followed by transcortical aphasia[4]. Cervical injury resulting in infarction of watershed areas may be due to kinking of vessels in the cervical region involving carotids and/or vertebral arteries. Atrophy of the hand was due to cervical injury[5] (site of lesion at C5-6 and C6-7) leading to LMN involvement of the hand muscles may be explained due to vascular congestion at C8,T1 below the level of injury[6].

Watershed infarction, though described in literature, is a rare association which should especially be thought of if a patient is having deranged sensorium or any neurological feature not pertaining to cervical injury[7].

References

1. Taneichi Hiroshi, Suda Kota, Kajino Tomomichi, Kaneda Kiyoshi. Traumatically Induced Vertebral Artery Occlusion Associated with Cervical Spine Injuries: Prospective Study Using Magnetic Resonance Angiography. *Spine* 2005; 30 (17): 1955-62.
2. Veras Luis M, Pedraza-Gutierrez Salvador, Castellanos J *et al*. Vertebral Artery Occlusion After Acute Cervical Spine Trauma. *Spine* 2000; 25 (9): 1171-7.
3. Akiyama K, Takizawa S, Tokuoka K *et al*. Shinohara bilateral middle cerebellar peduncle infarction caused by traumatic vertebral artery dissection. *Neurology* 2001; 56 (5): 693.
4. Julien B, Franco R, Gil A. Acute transcortical mixed aphasia a carotid occlusion synd.with pial and watershed infarcts. *Brain* 1988; 111 (3): 631-41.
5. Tamburin S, Zanette G. Focal hand dystonia after cervical whiplash injury. *Journal of Neurology, Neurosurgery and Psychiatry* 2003; 74: 134.
6. Schellinger Peter D, Schwab Stefan, Krieger Derk *et al*. Masking of Vertebral Artery Dissection by Severe Trauma to the Cervical Spine. *Spine* 2001; 26 (3): 314-9.
7. Zulch KJ. Uber die entstenhung und lokalisation der himinfarkte. *Acta Neurol Chir* 1961; 7 (Suppl): 1-117.

CASE REPORT

JIACM 2010; 11(1): 54-8

Small Vessel Vasculitis Associated with Myocardial Infarction

MP Agarwal, S Dwivedi**, SC Chaudhary***, S Khanna****, S Agarwal******

Abstract

Coronary vasculitis is an uncommon but catastrophic clinical presentation of small vessel vasculitis. We hereby report a case of leukocytoclastic vasculitis associated with myocardial infarction. Issues relating to thrombolytic therapy and coronary angiography in such a situation are discussed.

Keywords: Palpable purpura, myocardial infarction, vasculitis.

Introduction

Leukocytoclastic vasculitis, also known as allergic or small vessel vasculitis, is characterised by palpable purpura. Underlying aetiologies include drugs (e.g., antibiotics), infections (e.g., hepatitis C), and connective tissue diseases. Henöch Schönlein purpura (HSP) is a subtype of acute leukocytoclastic vasculitis that is seen primarily in children and adolescents following an upper respiratory tract infection. The majority of lesions are found on the lower extremities and buttocks. Systemic manifestations includes fever, arthralgias (primarily of the knees and ankles), abdominal pain, gastrointestinal bleeding and nephritis[1]. Coronary artery involvement is seen in vasculitic disorders like infectious angiitis, Takayasu's arteritis, granulomatous giant cell arteritis, thromboangiitis obliterans, polyarteritis nodosa, Wegener's granulomatosis and in Chürg Strauss syndrome[2]. However, there are infrequent case reports of HSP with myocardial infarction (MI) in adults[3,4].

Case report

A 67-year-old hypertensive male, on irregular treatment for the last one year presented with complaints of arthralgia (both ankles, knees, and wrist joints) of two days duration. Blood pressure recorded at that time was 200/120 mmHg. He was prescribed diclofenac gel, tab diclofenac, paracetamol, prednisolone, amlodipine, atenolol, losartan, hydrochlorothiazide, and atorvastatin (20 mg). Thereafter, he developed palpable purpura over both lower limbs, buttocks, lower abdomen and both upper limbs upto the elbow (Fig. 1). During this period he also suffered from acute retrosternal chest pain associated with diaphoresis (perspiration), and presented to the medical emergency. His pulse was 76 per minute and blood pressure was 150/90 mmHg. He was lean built, a non-smoker, and had no family history

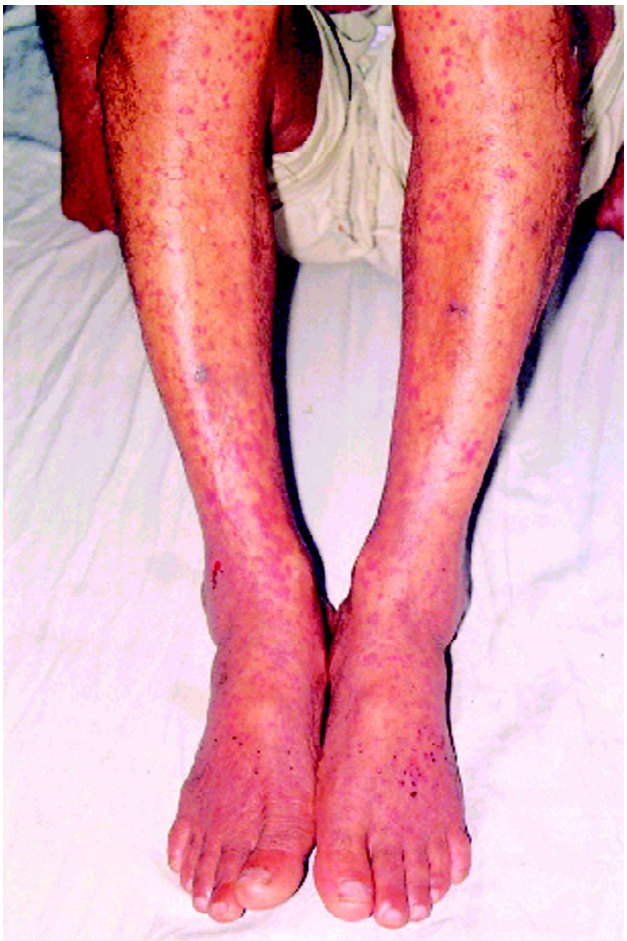


Fig. 1: *Palpable purpura seen over both lower limbs.*

** Professor, ** Professor and Head, *** Ex-Senior Resident, **** Postgraduate Student, Department of Medicine, ***** Professor and Head, Department of Pathology, University College of Medical Sciences, University of Delhi, and GTB Hospital, Shahdara, Delhi - 110 095.*

of hypertension, diabetes, sudden cardiac death, cardiovascular disease, or cerebrovascular accident. He denied any history of haematuria, bleeding from any other site, fever, or colicky abdominal pain. ECG showed changes suggestive of high lateral wall ST-elevated MI (Fig. 2) and cardiac troponin T (cTnT) was positive. Hess test and diascopy tests were negative and Buschke-Ollendorf sign was positive. Platelet count was within normal limits. A clinical diagnosis of anaphylactoid purpura, hypertension, and acute coronary syndrome (high lateral wall MI) was made. The patient was thrombolysed thereafter. The chest pain improved and ECG done a day after thrombolysis showed T wave inversion in lead I, avL, and V1-V6 (Fig. 3). Further investigations revealed positive ASLO titre. Elevated cardiac enzyme (CPK-MB - 30 ng/ml). His fasting lipid profile, fasting and post-prandial glucose levels, urine routine and microscopy, X-ray chest PA view were normal. HBsAg, anti-HCV, HIV, CRP, RA factor, C and P ANCA, and ANA were negative. Cutaneous biopsy revealed mild keratosis, and there was marked neutrophilic infiltration in and around the blood vessels suggestive of leukocytoclastic vasculitis (Fig. 4). Echocardiography revealed dilatation of aortic root, concentric left ventricular hypertrophy, E/A ratio 0.9, mild hypokinesia of free lateral wall with LVEF 63%. Coronary angiography to assess obstructive lesions was planned; however, the patient did not agree to this procedure.

Discussion

Palpable purpura is a cutaneous manifestation of several diseases. Although palpable purpura is the classic skin lesion of cutaneous vasculitis, it may also result from infection, malignancy, or embolic disease. Conversely, vasculitis may manifest as skin lesions, other than palpable purpura. Cutaneous vasculitis itself may result from several diseases that have a poorly understood pathogenesis.

The vasculitides presenting with palpable purpura are those involving small vessels (mainly post-capillary venules) and are characterised histologically by the term "leukocytoclastic vasculitis" (destruction of neutrophils with nuclear debris). This type of vasculitis may be referred to as "small-vessel vasculitis", "cutaneous vasculitis", or "hypersensitivity vasculitis". Combinations occur as in polyarteritis nodosa and Wegener's granulomatosis with both small- and medium-sized arteries being involved. These diseases also may present with leukocytoclastic vasculitis and palpable purpura. Symptoms associated with palpable purpura include pruritus, burning, and less often, pain. Lesions may be asymptomatic; however, so the presence or absence of these symptoms is not particularly helpful in the differential diagnosis.

Since one of the most likely causes of palpable purpura is small-vessel vasculitis, it is reasonable to approach the diagnosis with cutaneous vasculitis foremost in mind.

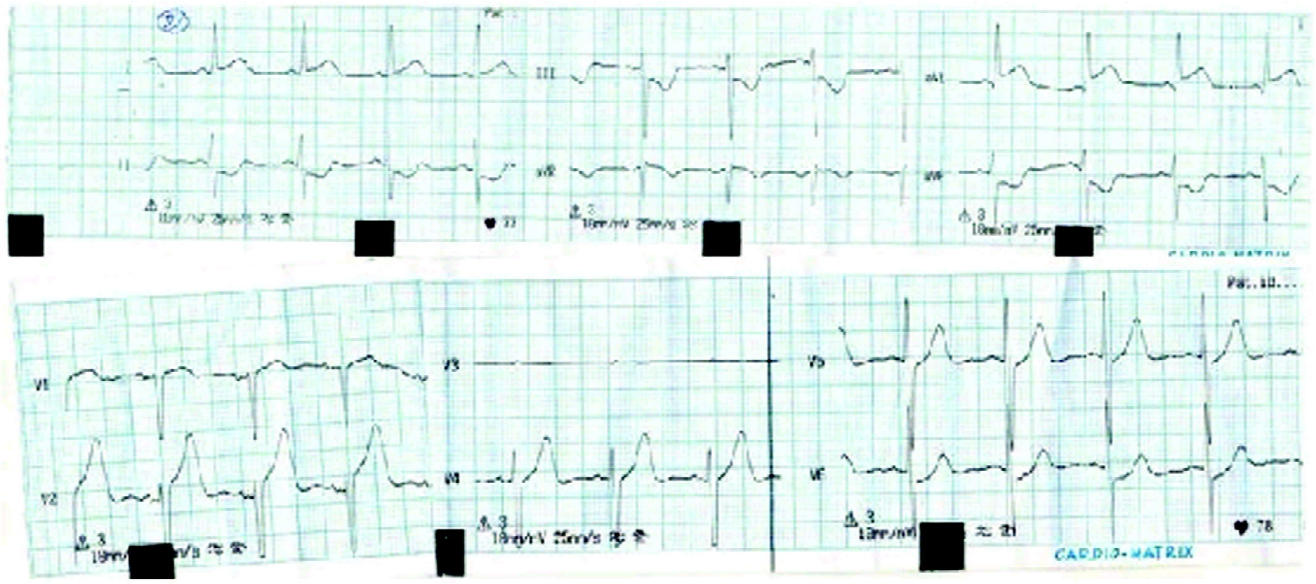


Fig. 2: *ECG showing ST elevation in leads I, avL, and ST depression in II-III and avF (pre-thrombolysis).*

Unless the cause is obvious from the clinical examination, an appropriate initial laboratory evaluation includes a complete blood count, erythrocyte sedimentation rate, blood cultures, antinuclear antibodies, and rheumatoid factor, blood urea nitrogen, serum creatinine, and urinalysis. An antineutrophil cytoplasmic antibody test with cytoplasmic fluorescence (c-ANCA can help make a diagnosis of Wegener's granulomatosis. The choice of

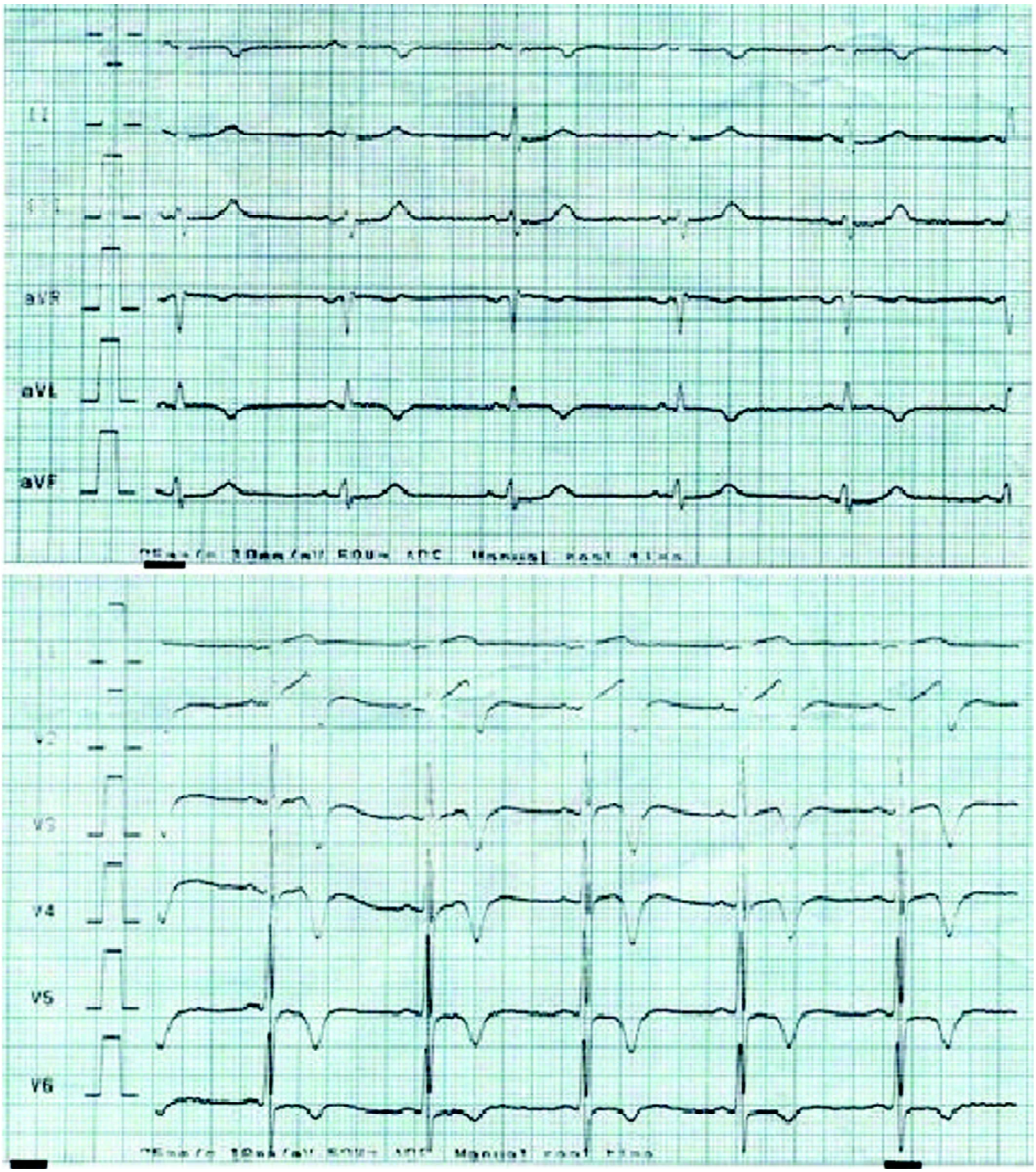


Fig. 3: *ECG showing T wave inversion in lead I, avL, and V1-V6 (post- thrombolysis).*

these tests is based on cost, the inflammatory nature of the disease process, and the most likely diseases associated with cutaneous vasculitis as reported in the literature. The sensitivity, specificity, and predictive value of these tests in patients with systemic disease should also be considered.

The patient's history may suggest a self-limiting cutaneous vasculitis, such as that resulting from a recent infection or drug reaction. In these cases, a skin biopsy is not necessary. However, a skin biopsy is warranted in patients with suspected vasculitis that is recurrent or longstanding, associated with systemic symptoms, or determined to be of unknown aetiology on the basis of the history and physical examination. In ambiguous cases, biopsy can help distinguish causes of palpable purpura other than vasculitis, such as cholesterol emboli because the condition is easily missed. Biopsy of cholesterol embolic lesions reveals arterioles occluded by fibrosis and multinucleated giant cells surrounding biconvex, needle-shaped clefts corresponding to dissolved cholesterol microemboli.

Unfortunately, biopsy of the skin may not provide a diagnosis of the specific vasculitis, since the cutaneous histopathology of the various vaculitides is often similar. Likewise, subacute bacterial endocarditis (SABE), Rocky Mountain spotted fever, and bacteraemia, involve immune complex deposition resulting in microscopic necrotising vasculitis, so clinical correlation is critical. Additionally, the inflammatory infiltrate of cutaneous vasculitis is dynamic, yielding a different picture depending on the time of biopsy. Because the infiltrate may progress from the characteristic neutrophil invasion of vessel walls with leukocytoclastis to a mononuclear infiltrate over the course of one or two days, it is recommended that the biopsy be performed within one to two days of the appearance of a given lesion to attain a specific diagnosis of cutaneous vasculitis[5].

In view of the history of drug intake preceding the appearance of palpable purpura, in our patient it appears

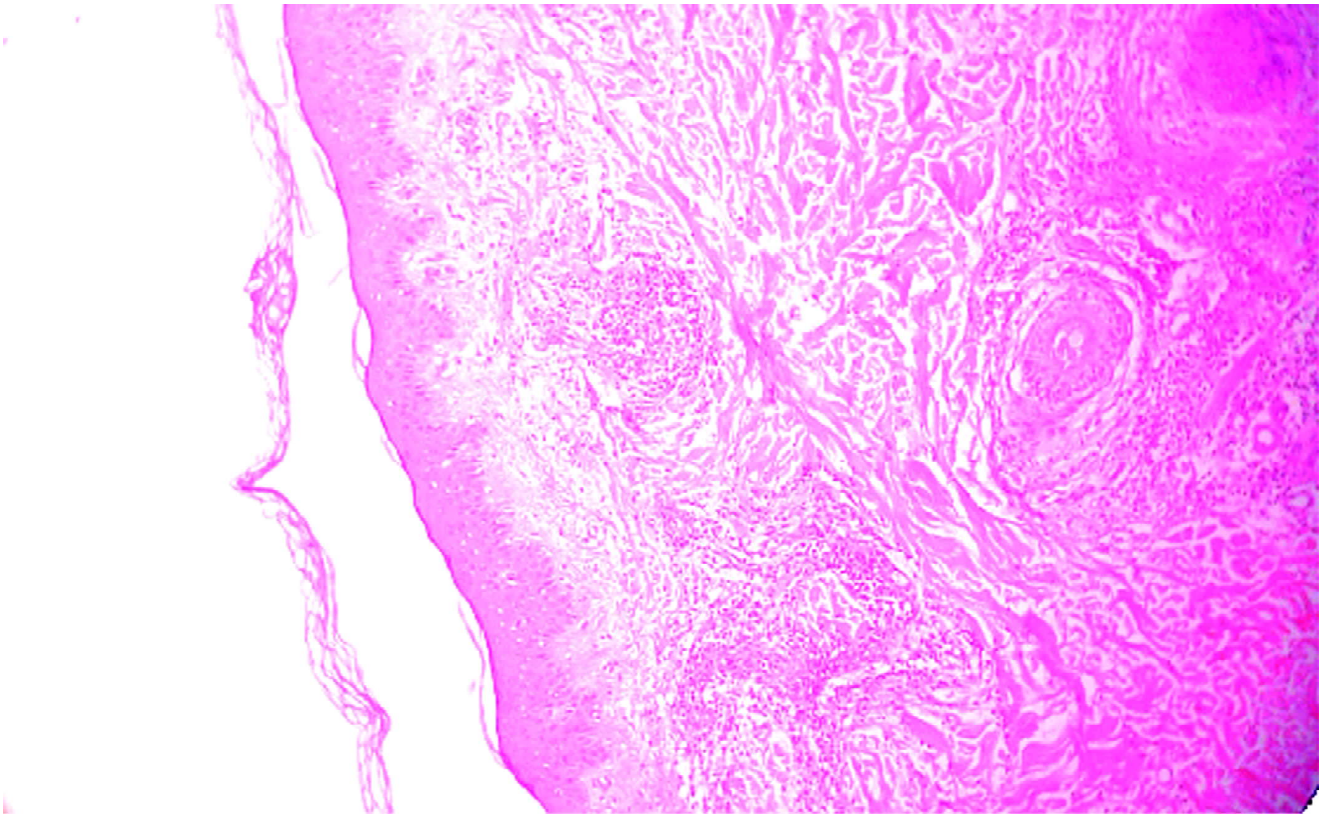


Fig. 4: *Histpathological section of cutaneous biopsy revealed marked neutrophilic infiltration in and around the blood vessels suggestive of leukocytoclastic vasculitis (H and E).*

that these rashes were drug-induced small vessel vasculitis. It thus appear to be an interesting association of small vessel vasculitis associated with ST elevated myocardial infarction warranting thrombolytic therapy. Considering normal platelet count and absence of bleeding from any sites, it was considered safe to give him the benefit of thrombolysis and low molecular weight heparin therapy to which the patient responded very well without any complications.

In patients with clinical, electrocardiographic, or laboratory signs of acute coronary syndrome, especially in absence of smoking, positive family history, obesity, or diabetes – the conventional risk factors for atherosclerosis – one should always look for non-atherosclerotic causes of myocardial infarction. Although this patient had antecedent hypertension, associated coronary vasculitis was considered plausible. Associated atherosclerotic coronary artery disease could not be definitely excluded for want of coronary angiography as the patient refused this procedure.

References

1. Bolognia JL, Braverman IM. Skin manifestations of internal disease. In: Kasper DL, Braunwald E, Fauci AS *et al*, editors. Harrison's Principles of Internal Medicine, 16th ed. New York: McGraw Hill; 2005; 310.
2. Stvrtinova V. Vasculitides of the coronary arteries. *Bratisl Lek Listy* 1995; 96: 544-51.
3. Satoh M, Mikuniya A, Mikami M *et al*. A case of Henöch-Schönlein purpura with myocardial complications. *Kokyu To Junkan* 1991; 39: 273-7.
4. Abdel-Hadi O, Greenstone MA, Hartley RB, Kidner PH. Myocardial infarction – a rare complication in Henöch-Schönlein purpura. *Postgrad Med J* 1981; 57: 390-2.
5. Stevens GL Adelman HM, Wallach PM. Palpable purpura: an algorithmic approach. *Am Fam Physician* 1995; 52: 1355-62.

CASE REPORT

JIACM 2010; 11(1) : 59-60

Splenic Infarct in Acute Malaria

OP Jatav*, Navneet Agrawal**, Dharmendra Tiwari**, Ravi Kiran***

Abstract

Malaria is endemic in India with a great socio-economic burden on the humanity. Plasmodium falciparum is associated with serious and fatal complications like cerebral malaria, acute renal failure, ARDS, hepatitis, haematological and coagulation abnormalities, etcetera. We are reporting a case of splenic infarct in acute falciparum malaria which is an uncommon complication.

Keywords: Splenic infarct, falciparum malaria.

Introduction

Malaria is the most important parasitic disease of man. Approximately 5% of the world's population is infected, and there are approximately one million deaths each year. Severe malaria has been a major cause of mortality worldwide, and *Plasmodium falciparum* is the main species for most causes of death. Among the known complications of malaria, splenic infarct is a rare complication[1]. Here we report a case of splenic infarct complicating acute malaria.

Case report

A 28-year-old female was admitted to the medical ward of our hospital with a 5-days history of fever which was moderate to high grade, intermittent, and associated with chills. She also had pain in the upper abdomen which was dull aching, at times severe, and was accompanied by vomiting. There was no history of cough, burning micturition, or bowel complaints at the time of admission.

On examination, the patient was haemodynamically stable and afebrile. There was no icterus, cyanosis, clubbing, pedal oedema, or lymphadenopathy. Examination of the abdomen revealed tender splenomegaly 3 cms below the left costal margin, and non-tender hepatomegaly 3 cms below right costal margin. Splenic rub was absent. Ascites was present. Examination of other systems was unremarkable.

During the course of hospitalisation, the patient developed non-productive cough. Chest examination revealed late inspiratory crepts in the left lower lung field.

Investigations revealed Hb - 7.4 gm%, WBC - 4,100/cumm, P65%, L 28%, E - 06%, M - 01%, total serum bilirubin - 1.29 mg/dl, SGPT - 23.94 IU, blood urea - 30.6 mg/dl, serum creatinine - 0.86 mg/dl. Peripheral blood smear revealed ring stage of *Plasmodium falciparum*, and normocytic-normochromic anaemia. Initial ultrasound study of the abdomen reported hepatosplenomegaly (spleen - 17.0 cms), and splenic infarct with moderate ascites. Repeat study after three days showed, in addition, minimal fluid collection in the sub-phrenic space with minimal left pleural effusion, and minimal ascites. The findings of ultrasound were confirmed by CT scan of abdomen (Fig. 1).

During her stay in the hospital, the patient was given inj. quinine, antibiotics - metronidazole, augmentin, amikacin. Inj. tramadol was given to relieve her abdominal pain. Two units of blood were also transfused. With clinical improvement in the condition of the patient, she was

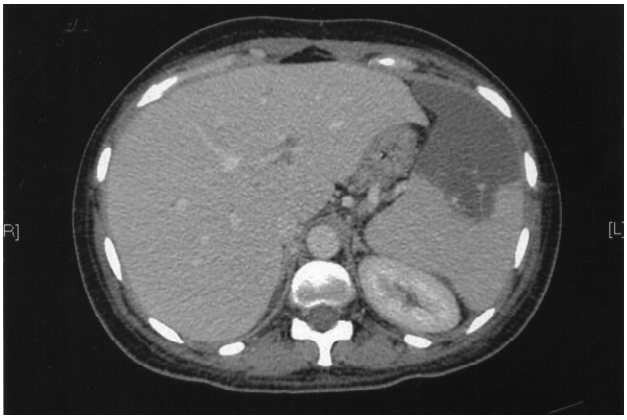


Fig. 1: *CT scan of abdomen showing splenic infarct.*

** Professor and Head, ** Assistant Professor, *** Resident, Department of Medicine, G. R. Medical College, Gwalior - 474 001, Madhya Pradesh.*

discharged subsequently. Follow-up ultrasound abdomen and chest X-ray two weeks after the discharge showed clearing of the pleural effusion and ascites with reduction in the enlarged splenic size.

Discussion

India is a highly endemic country for malaria. Often patients present to the hospital with the complications and features of severe malaria like cerebral malaria, renal failure, shock, bleeding manifestations, hypoglycaemia[2].

Our patient had severe pain in the left upper quadrant of the abdomen which on ultrasound study and CT scan of the abdomen was confirmed to be due to a splenic infarct.

Splenic infarct is a known but a rare complication of acute malaria[2]. To the best of our knowledge, only nine documented cases of splenic infarction associated with malaria have been reported, of which six were attributed to *Plasmodium falciparum* infection, one was co-infected with *Plasmodium vivax*, and in two cases the aetiology was not known[3]. Other common causes of splenic infarct were excluded by studying the peripheral blood smear and coagulation profile[4]. The spleen plays a central role in limiting the acute expansion of the malaria infection by removing parasitised erythrocytes. Splenic enlargement occurs in 95 – 100% of individuals with acute malaria. During acute and chronic malaria, altered splenic structure and function results in mild-to-moderate enlargement of the spleen. The possible factors contributing to splenic infarction in falciparum malaria are tissue avascularity following rapid splenomegaly and increased stickiness of the parasitised RBCs[4]. It requires high degree of suspicion to diagnose the condition, and in places where investigations like USG and CT scan are sparsely available, the diagnosis may be missed.

Our patient also developed cough during hospitalisation as a result of left-sided pleural effusion which was reactionary to splenic infarct[5]. Ascites in this patient may be reactionary to infarct as it subsided subsequently.

The mainstay of medical therapy of splenic infarct is analgesia with either narcotics or non-steroidal anti-inflammatory agents, and prevention of secondary infection. The treatment of splenic infarct does not involve active intervention but meticulous observation to look for its complication and supportive treatment. Complications include haemorrhage, subphrenic abscess, pancreatic fistula, and gastric fistula. Splenic abscess results from septic emboli or super-infection of a prior infarct[6]. The above complications warrant surgical intervention[7].

Though the complications of splenic infarct due to other causes are documented, it is yet to be reported in cases occurring due to acute malaria. Hence, in the malaria endemic areas like ours, it should be considered in the differential diagnosis of the patients presenting with upper abdominal pain and fever.

References

1. Hovette P, Lecoules S, Boete F *et al*. Splenic infraction during *P. falciparum* and *P. vivax* malaria. *Presse Med* 1994; 23: 1226.
2. World Health Organisation. World Malaria situations in 1993. *Wkly Epidemiol Rec* 1996; 71: 17-24.
3. Bonnard P, Guiard-Schmid JB, Eveloux M *et al*. Splenic infarction during acute malaria. *Trans R Soc Trop Med Hyg* 2005; 99: 82-66.
4. Guth AA, Pachter HL. Splenic infarct. *eMedicine J* 2002; 3 (4): 1-11.
5. Maza Leitersdorf E. Splenic infarction as an unusual cause of left pleural effusion IB, Amital H, Barkan D, Shaham D, PMID: 15523806.
6. Gupta S, Kakar A. Splenic Infarct of Unusual Aetiology. *JIACM* 2004; 5 (4): 310-4.
7. Nores M, Philleps EH, Morgenstren L. The clinical spectrum of splenic infarction. *Ann Surg* 1998; 64 (2): 182-8.

CASE REPORT

JIACM 2010; 11(1): 61-2

Tuberculous Meningitis with Blindness

Umesh Das, Vishal Kumar Gupta*, Pushpa Yadav**, Vipin Mediratta***, BB Rewari***, SC Sharma***

A 22-year-old married female presented with gradual onset weakness of the right side of the body since the last one year, and also inability to speak and loss of vision for the same duration. She was diagnosed as tubercular meningitis one-and-a-half year back, and now was on anti-tubercular drugs under DOTS category II. Her general physical examination was normal. Neurology examination revealed that she was mentally retarded with an IQ of 35, and global aphasia. There was right-sided hemiparesis, power being 3/5 with exaggerated DTJ, and plantar extensor on the right side. Cranial nerve examination revealed that she was totally blind with absent finger counting for both eyes, and absence of projection of rays and perception of light. Fundus examination revealed bilateral secondary optic atropy with normal foveal response. Complete blood count including ESR was normal. ANA, rheumatoid factor, VDRL, and HIV tests were negative. CSF showed cell count of 250/cumm, predominantly lymphocytes, protein was 47 mg% and sugar was 65 mg%. Visual evoked potential revealed no response in both the eyes and bilateral visual pathway dysfunction. MRI brain showed panencephlomalacia in the left temporo-parietal region (Fig. 1). Caseous granulomatous lesions seen in suprachiasmatic region (Fig. 2) and over the right cerebellar region hemisphere (Fig. 3). Similar lesions

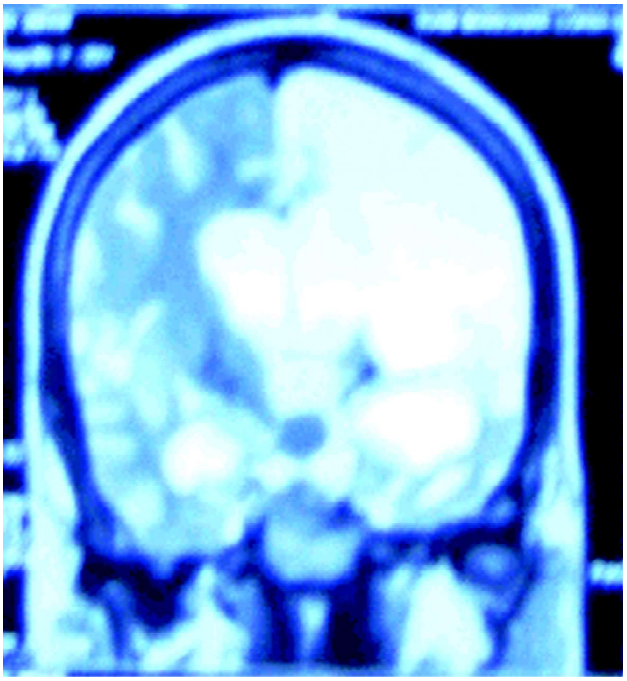


Fig. 1: *MRI of brain showing panencephalomalacia in the temporo-parietal region.*

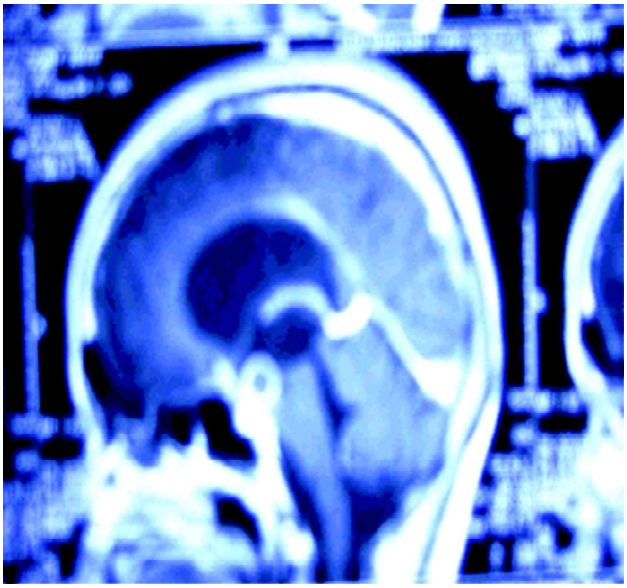


Fig. 2: *MRI showing caseous granulomatous lesions in suprachiasmatic region.*

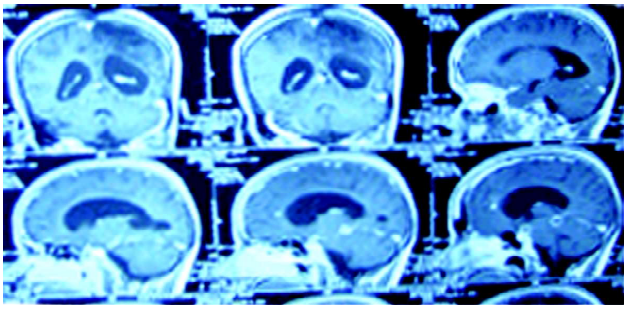


Fig. 3: *MRI showing caseous granulomatous lesions over the right cerebellar region hemisphere.*

** Senior Resident, ** Consultant, *** Physician, Department of Medicine,*
PGIMER, Dr Ram Manohar Lohia Hospital, Baba Kharak Singh Marg, New Delhi - 110 001.

were seen in the right and left inferior frontal lobe. Bilateral optic nerves were of normal morphology, intensity, and course (Fig. 4). Lateral ventricles and 3rd ventricle were dilated, 4th ventricle was grossly normal, suggestive of communicating hydrocephalus. There was evidence of pachymeningitis.

Tuberculosis is still widely prevalent in India. With modern therapy, the survival rate in tuberculous meningitis is now increasing. As a result, neuro-ophthalmic complications which were less common due to early mortality several years ago, are now reasonably frequent. Although tuberculoma of the central nervous system (CNS) is rare in developed countries, in developing countries such as India, the incidence of tuberculoma is as high as 20%[1].

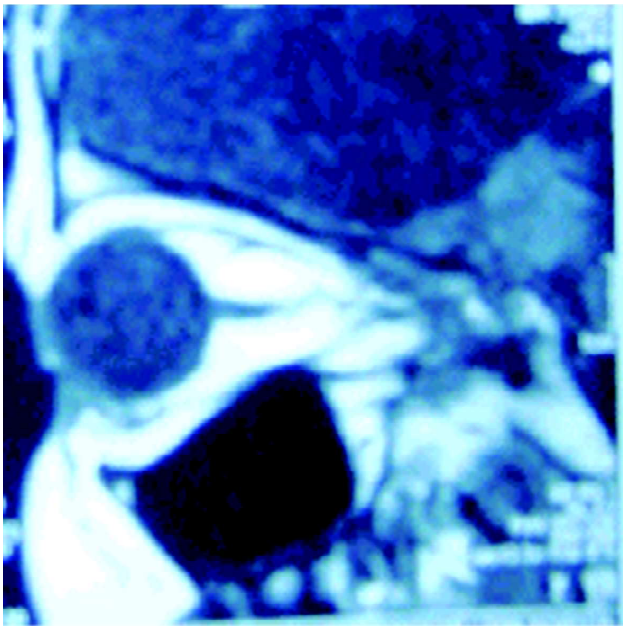


Fig. 4: *MRI showing normal optic nerves.*

A high incidence of ophthalmologic abnormalities occur in patients with intracranial tuberculomas, even though the lesions do not directly involve the central visual pathways. The most common abnormalities are extraocular motor nerve palsies, visual field defects, and impaired visual acuity, all of which are usually secondary to increased intracranial pressure[1]. Papilloedema was found in 34% of cases[2]. Presence of papilloedema indicates obstruction to CSF outflow and consequent rise in intracranial pressure. Ocular palsy was found in 20% cases. Other associated focal neurological signs were seen in 22% cases. This was a late complication of the disease and was usually associated with tuberculomas[3].

Blindness in one or either eye was rare. Compression of the visual pathways by tuberculoma, tubercular arachnoiditis, vascular insufficiency, and prolonged rise in intracranial pressure, or arachnoidal adhesions, were the common aetiological factors[4, 5]. In our case, the blindness was due to compression of the optic nerve chiasma by a tuberculoma.

Acknowledgment

We are grateful to the department of Radiology, Dr Ram Manohar Lohia Hospital for their valuable help.

References

1 Schlernitzauer DA, Hodges III FJ, Bagan M. Tuberculoma of the Left Optic Nerve and Chiasm. *Arch Ophthalmol* 1971; 85 (1): 75-8.

2 Thomas MD, Chopra JS, Banerjee AK, Singh MS. Tuberculous meningitis. A clinico-pathological study. *Neurology India* 1977; 25: 27.

3 Misra M, Rath S, Acharya BN *et al*. Neuro-ophthalmic profile in tubercular meningitis a clinico-radiological correlation. *Ind J Tub* 1985; 23: 142.

4 Wadia NH, Singhal BS. Cerebral angiography in tuberculous meningitis. A study of 33 cases. *Neurology India* 1967; 15: 127.

5 Rath S, Panda DK, Biswas AC. Arteriographic study in tuberculous meningitis. *Phronesis (Oviedo-Spain)* 1972; 4: 44.

CASE REPORT

JIACM 2010; 11(1): 63-5

Macrophage Activation Syndrome

Ashok Duggal, Gurminder Kaur***

Abstract

Macrophage activation syndrome or haemophagocytic lymphohistiocytosis (HLH) is a heterogeneous group of disorders characterised by activated macrophage activity leading to a sepsis-like clinical presentation with haemophagocytosis, hyperferritinaemia, hypercytokinaemia, and variable cytopenias often resulting in multiple organ failure.

Introduction

Macrophage activation syndrome is a rare disorder with typical histology showing tissue infiltration by lymphocytes and macrophages, some manifesting haemophagocytosis. The term haemophagocytosis describes the pathologic finding of activated macrophages engulfing erythrocytes, leukocytes, platelets, and their precursor cells[1]. It appears likely that HLH is due to the abnormal function of T lymphocytes. Tissue infiltrates and haemophagocytosis is probably due to the dysregulated secretion of cytokines, rather than by a primary disorder of macrophages. The disorder occurs in primary and secondary forms. Primary HLH is an autosomal recessive disorder. Secondary HLH is at least as common as primary disease. Precipitants include viral, bacterial, fungal, or protozoan infections, often in an immunocompromised host[2-6]. Other precipitants include malignancy, particularly T-cell lymphoproliferative states, autoimmune diseases, and lipid infusions.

This case is presented with the aim to refresh the knowledge of physicians regarding the clinical entity 'macrophage activation syndrome' which is to be suspected when patients present with fever unresponsive to antibiotics, falling ESR, pancytopenia of unknown aetiology, and splenomegaly with elevated ferritin levels[7,8].

Case history

A 24-year-old female was admitted to our institution with the history of high-grade fever (104° - 105°F), and headache associated with vomiting of 5 days duration. Patient was a known case of epilepsy for the last three years and was taking phenytoin irregularly.

On examination, she was conscious, febrile (104°F) having arterial pressure of 110/70 mmHg, pulse - 118/min, respiratory rate - 24/min. She had no cyanosis, no jaundice, no clubbing, no pedal oedema, no significant lymphadenopathy. Patient had mild splenomegaly but no hepatomegaly. During chest examination, no significant abnormality was detected. Examination of the heart revealed no abnormality except for tachycardia. There was no sign of meningeal irritation, no focal neurological deficit, and normal fundus.

Investigations revealed

Hb - 7.6 gm%; TLC - 7,400/cmm; DLC - P_{70}, L_{26}, E_2, M_2; ESR - 125 mm/1st hr, platelet count - 70,000/cmm. Peripheral smear report showed pancytopenia and no malarial parasite.

A bone marrow aspiration study revealed hypocellular marrow.

Biochemical examination revealed: RBS - 105 mg%; blood urea - 24 mg%, and creatinine - 1.2 mg%, bilirubin - 1.2 mg%, SGOT/SGPT 86/88, PTI - 93%, serum electrolytes were sodium - 136 mEq/l, and potassium - 3.8 mEq/l. G-6PD was normal.

Urine examination showed many RBCs and a few pus cells. Urine and blood culture showed no growth.

Rheumatoid factor, viral markers for human immunodeficiency virus, hepatitis B, C, and serology for dengue, widal, rapid malarial test were negative and chest X-ray revealed no significant pathology. Sonogram of abdomen revealed mild splenomegaly. ECG showed sinus tachycardia. Echo showed no evidence of infective

** Assistant Professor, ** Resident, Department of Medicine, Government Medical College, Amritsar, Punjab.*

endocarditis and normal systolic function.

The patient was administered antibiotics, and blood transfusions were also given, but she never responded. Inspite of repeated blood transfusions, she continued to have high-grade fever which never touched the baseline and required repeated hydrotherapy.

On repeat investigations, the patient's cell counts declined further: TLC declined from 7,400 to 3,000 cells/cumm, platelets to 40,000/cumm, ESR to 88 mm in the 1st hr. PTI declined from 92% to 73%, whereas SGOT/SGPT were found to be raised with a level of 132/126 IU/l.

After this, fresh blood transfusions were given, and anti-epileptics were tapered off fearing drug induced aplastic anaemia.

The clinical scenario aroused the suspicion of macrophage activation syndrome. To confirm the diagnosis serum ferritin levels, triglyceride, and LDH were ordered.

Ferritin was elevated [3,234 ng/ml; (0 – 150 ng/ml)]. Triglycerides were 180 mg%, serum LDH was 461. Repeat bone marrow examination resulted in a dry tap, following which bone marrow biopsy was ordered and the patient was advised a course of IV immunoglobulins. But the bone marrow biopsy could not be undertaken as the patient left the hospital and preferred to go to the military hospital due to the economic constraints where her condition deteriorated further and she started having epistaxis and oropharyngeal bleeding. She died 20 – 25 days later.

Discussion

Macrophage activation syndrome (MAS), or haemophagocytic lymphohistiocytosis (HLH) is a rare and potentially fatal disease of normal but overactive histiocytes. It is of two types – primary HLH, and secondary HLH (acquired HLH) which occurs after strong immunologic activation – systemic infection (virus, bacteria, and protozoa), autoimmune disorders, or an underlying malignancy.

The clinical entity has to be suspected when patients present with fever unresponsive to appropriate antibiotics, falling ESR, pancytopenia of unknown origin, and liver dysfunction with elevated ferritin and LDH levels. The diagnostic criteria proposed by the Histiocyte Society for inclusion in the International Registry for Haemophagocytic lymphohistiocytosis is as follows[9]:-

1. Fever – seven or more days of a temperature as high as 38.5°C (101.3°F).
2. Splenomegaly.
3. Cytopenia – counts below the specified range in at least two of the following cell lineages:
   - I. Absolute neutrophils less than 1,000/ml;
   - II. Platelets less than 100,000/ml;
   - III. Haemoglobin less than 9.0 g/dl.
4. Hypertriglyceridaemia (> 160 mg/dl), or hypofibrinogenaemia.
5. Hyperferritinaemia (serum ferritin > 1,000 ng/ml).
5. Haemophagocytosis (in bone marrow or RE tissues).
6. Rash.

At least five of them have to be there to have a definite diagnosis. For confirmation, tissue diagnosis is needed. Haemophagocytosis is usually demonstrated in the bone marrow, spleen, or lymph nodes, but haemaophagocytes may not be present in the bone marrow in early stages when the process may either be seen in other tissues (spleen or enlarged lymph nodes). Findings in upto two-thirds of initial bone marrow aspirates may be nondiagnostic; thus, a negative examination finding may not rule-out HLH. An additional bone marrow finding includes dyserythropoiesis, which has been observed in the absence of haemophagocytic histiocytes. Additional studies, including lymph node biopsy, should be undertaken, and treatment should not be delayed if all other criteria have been met. Although problematic in a patient with a coagulopathy, a liver biopsy demonstrating a picture similar to chronic persistent hepatitis can support the diagnosis, as can the presence of mononuclear cells in the cerebrospinal fluid.

Treatment

Two different types of agents can be used:

1. To interrupt the function of activated macrophages and histiocytes, i.e., corticosteroids, high-dose immunoglobulins and aetoposide.
2. To interrupt the function of activated T-lymphocytes, i.e., corticosteroids, antithymocyte globulins, immunosuppressive agents like cyclosporine A and cyclophosphamide.

Plasma exchange is helpful in improvement of hypercytokinaemia.

Initial therapy consists of aetoposide and dexamethasone for 8 weeks in varying doses. In the HLH-2004 protocol, cyclosporine is added in the beginning. Intrathecal methotrexate is used only with persistently abnormal CSF or progressive neurologic symptoms[10-11]. HLH associated with malignancies demands prompt therapy directed at the neoplasm.

Cyclosporin A appears to switch off the immune reaction in early stages and is considered to be the drug of choice[12].

Conclusion

High index of suspicion is needed to make a diagnosis of macrophage activation syndrome. A patient with high swinging fever, pancytopenia, hepatic enzyme abnormalities should arouse the suspicion of MAS. Once suspected, LDH and ferritin level along with bone marrow aspiration should be done. An aggressive treatment schedule including appropriate antibiotics, corticosteroids, IV immunoglobulins, immuno-suppressive agents can be used with varying success rates.

References

1. Favara B. Hemophagocytic lymphohistiocytosis: a haemophagocytic syndrome. *Semin Diagn Pathol* 1992; 9: 63-74.
2. Onishi R, Namiuchi S. Haemophagocytic syndrome in a patient with rheumatoid arthritis. *Intern Med* 1994; 33: 607-11.
3. Wong KF, Hui PK, Chan JK *et al*. The acute lupus haemophagocytic syndrome. *Ann Intern Med* 1991; 114: 387-90.
4. Kumakura S, Ishikura H, Munemasa S *et al*. Adult onset Still's disease associated haemophagocytosis. *J Rheumatol* 1997; 24: 1645-8.
5. Morris JA, Adamson AR, Holt PJ, Davson J. Still's disease and the virus-associated haemophagocytic syndrome. *Ann Rheumatol Dis* 1985; 44: 349-53.
6. Yasuda S, Tsutsumi A, Nakabayashi T *et al*. Haemophagocytic syndrome in a patient with dermatomyositis. *Br J Rheumatol* 1998; 37: 1357-8.
7. Koduri PR, Carandang G, DeMarais P, Patel AR. Hyperferritinaemia in reactive haemophagocytic syndrome: report of four adult cases. *Am J Hematol* 1995; 49: 247-9.
8. Esumi N, Ikushima S, Hibi S *et al*. High serum ferritin level as a marker of malignant histiocytosis and virus-associated haemophagocytic syndrome. *Cancer* 1987; 61: 2071-6.
9. Henter JI, Elinder G, Ost A. Diagnostic guidelines for haemophagocytic lymphohistiocytosis. *Semin Oncol* 1991; 18: 29-33.
10. Henter JI, Arico M, Elinder G *et al*. Familial haemophagocytic lymphohistiocytosis. Primary haemophagocytic lymphohistiocytosis. *Hematol Oncol Clin North Am* 1998; 12: 417-33.
11. Imashuku S, Hibi S, Ohara T *et al*. Effective control of Epstein-Barr virus-related haemophagocytic lymphohistiocytosis with immunochemotherapy. *Blood* 1999; 93: 1869-74.
12. Oyama Y, Amano T, Hirakawa S *et al*. Haemophagocytic syndrome treated with cyclosporin A: a T-cell disorder? *Br J Haematol* 1989; 73: 276-8.

ACKNOWLEDGEMENT

LIST OF *JIACM* PRIME REVIEWERS (2009)

- Alladi Mohan (Tirupati)
- Anil Mahajan (Jammu)
- B.O. Toyade (Nagpur)
- Geeta Khwaja (New Delhi)
- O.P. Kalra (New Delhi)
- R.S.K. Sinha (New Delhi)
- Rajat Kumar (Canada)
- Rajesh Khadgawat (New Delhi)
- Rohini Handa (New Delhi)
- Vimlesh Purohit (New Delhi)

CASE REPORT

JIACM 2010; 11(1): 66-8

Novel Pulmonary Presentation of *Mycobacterium interjectum*

Adam W Bagley, Jaspal S Gujral***

Abstract

Non-tuberculous mycobacteria (NTM) have been shown to cause disseminated disease in immunocompromised individuals as well as local infection in the immunocompetent. In 2006 the incidence of NTM disease varied from 1.0 to 1.8 cases per 100,000 in industrialised countries. The number of NTM species and diagnoses have increased dramatically as a result of improved isolation techniques and sequencing of the 16S rRNA as a standard for identifying new species. The NTM known as Mycobacterium interjectum has been well-documented as a source of infection in children and immunocompromised adults; however, there are no descriptions of disease in healthy individuals. To date, no human-to-human transmission has been described, and infections are believed to be acquired from environmental exposure. The following is a description of significant pulmonary disease in an immunocompetent gentleman with M. interjectum as the identified pathogen.

Key words: Non-tuberculous mycobacteria, chest wall abscess, Mycobacterium interjectum.

Introduction

Nontuberculous mycobacteria (NTM) have been shown to cause disseminated disease in immunocompromised individuals as well as local infection in the immunocompetent. In 2006 the incidence of NTM disease varied from 1.0 to 1.8 cases per 100,000 in industrialised countries. The number of NTM species and diagnoses have increased dramatically as a result of improved isolation techniques and sequencing of the 16S rRNA as a standard for identifying new species. The NTM known as *Mycobacterium interjectum* has been well documented as a source of infection in children and immunocompromised adults; however, there are no descriptions of disease in healthy individuals. To date no human-to-human transmission has been described, and infections are believed to be acquired from environmental exposure.

Case report

A 56-year-old white male presented with pain in the right axilla of two months duration. He was initially diagnosed via CT scan at another hospital with an infection of the lung parenchyma but this was not seen on a repeat scan at yet another hospital. Increasing pain, waking the patient from sleep prompted his presentation. He described a nonproductive cough of six months duration, subjective fever, and dyspnoea on exertion. The patient was taking no medications and has no history of chronic obstructive pulmonary disease (COPD). Past medical history is significant for pneumonia as a child, tobacco abuse of fifteen pack-years, and *Mycobacterium Avium Complex* (MAC) of the left lung that was treated and lost to follow-up eleven years prior.

The patient was afebrile with stable vitals and an oxygen saturation of 90% on room air. Examination revealed coarse breath sounds bilaterally. The right chest wall and axilla were diffusely tender and demonstrated crepitus on palpation. No erythema, ulceration, or lymphadenopathy was noted.

Laboratory data included: Hb: 11.7 g/dl, Hct: 34.5%, WBC: 11,000 cells/µl, platelets: 719,000 cells/µl, MCV 84.2 µm^3, normal basic metabolic panel, and normal liver enzymes. Blood cultures, HIV, and tuberculin skin tests were negative. Iron studies revealed: iron level: 18 µg/dl, Ferritin: 99.1 ng/ml, total iron binding capacity: 225 µg/dl, iron saturation: 7%, and WESR: 90 mm/hr. These values indicated a chronic inflammatory process, thus thrombocytosis was classified as reactive.

CT scan demonstrated bilateral cavitary lesions of the lung apices (Fig. 1), as well as inflammation and an infectious process of the right lung with extension of infection through the anterior lateral chest wall with a small abscess and air collection posterior to the pectoralis.

** Junior Medical Student, ** Associate Professor, Department of Medicine, Medical College of Georgia, Augusta, GA 30912, USA.*

Aspiration of the fluid collection recovered 4 ml of thick bloody purulent material that was sent for cytology and microbiological analysis (Fig. 1). Three sputum samples taken on different days, bronchioalveolar lavage, and the aspirated fluid were each found to contain acid-fast bacteria.

The patient was discharged with a presumptive diagnosis of recurrent MAC, and prescribed ethambutol 1,200 mg daily, rifampin 300 mg daily, and clarithromycin 500 mg twice daily with instructions to follow-up with pulmonary medicine.

After discharge, cultures of the aspirate for nocardia and actinomycetes, cytology, and blood cultures, were all negative. Mycobacterium samples are analysed, and if a diagnosis of MAC or *M. tuberculosis* is not made, sequencing of 16S rRNA is performed. Sputum cultures, bronchioalveolar lavage, and aspirate were all found to contain *Mycobacterium interjectum* as identified by DNA sequencing.

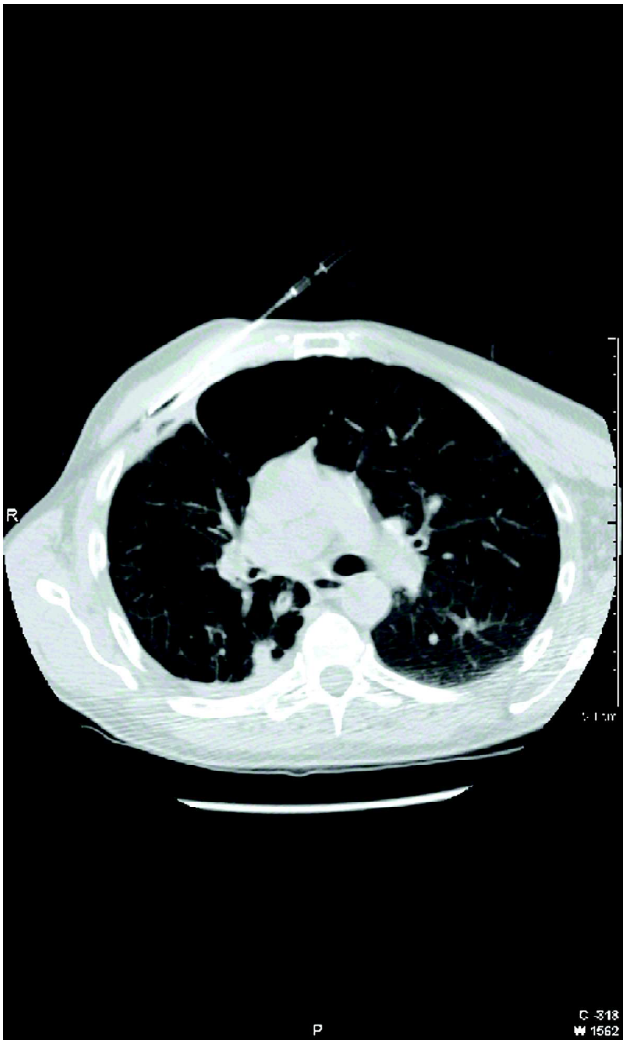


Fig. 1: *Extensive cavitary lesions of both upper lobes with soft-tissue density outlining right upper lobe cavities and CT directed aspiration of right anterior chest wall air-fluid collection.*

Discussion

The number of non-tuberculous mycobacteria (NTM) species and diagnoses have increased dramatically as a result of improved isolation techniques and sequencing of the 16S rRNA as a standard for identification of NTM species[1]. *Mycobacterium interjectum* was first identified in 1993 in a child with chronic lymphadenitis[2]. Fifteen cases have been reported, nine of which are associated with lymphadenitis in children aged one to three[3, 4]. Of the six cases in adults, the only case considered clinically significant is a 52-year-old female with severe COPD and multiple positive cultures[5]. Of note, there are no previous documented infections in immunocompetent healthy adults.

The bacterium presented in a novel fashion with crepitus and pain from lung and soft tissue damage in an apparently healthy 56-year-old male. The American Thoracic Society has set stringent criteria to diagnose NTM infection, the patient exceeds these requirements meeting each possible criterion[1].

As no features of cutaneous infection were present, we believe that the bacterium eroded lung tissue to the extent of forming an abscess deep to the pectoralis muscle allowing air to escape into soft tissues. After reviewing imaging and physical exam findings, it was determined that the axillary pain was the result of pectoralis inflammation.

We theorise that childhood pneumonia, which requiried hospitalisation, left the lungs scarred allowing colonisation with the mycobacterium. Tobacco could be an additional risk factor, though there are minimal radiological and clinical signs of COPD, and this would not explain the diagnosis of MAC eleven years prior. Genetic factors such as interferon-γ or interleukin-12 deficiencies were also considered, however these would predispose to disseminated disease[1]. We also speculate the previous diagnosis of MAC was

actually *M. interjectum*, and as the patient failed to follow-up, the bacterium was never cleared.

Conclusion

To our knowledge this is the first description of *M. interjectum* as a virulent pathogen in a non-immunocompromised healthy adult. While the source of infection as well as why virulence is noted in this individual can only be hypothesised, it is clear that *M. interjectum* can act as a human pathogen producing significant disease. As more laboratories adopt 16S rRNA gene sequencing for NTM identification, further descriptions of this and other NTM species as pathogens are likely in the future.

Acknowledgements

We would like to thank David Webb, MD, and Clarence Joe, MD for their help in preparing this case report.

References

1. Griffith D, Aksamit T, Brown-Elliott B *et al*. An official ATS/IDSA statement: diagnosis, treatment, and prevention of non-tuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2006; 175 (4): 367-416.
2. Springer B, Kirschner P, Rost-Myer G *et al*. *Mycobacterium interjectum*, a new species isolated from a patient with chronic lymphadenitis. *J Clin Microbiol* 1993; 31 (12): 3083-9.
3. De Baere T, Moerman M, Rigouts L *et al*. *Mycobacterium interjectum* as causative agent of cervical lymphadenitis. *J Clin Microbiol* 2001; 39 (2): 725-7.
4. Fukuoka M, Matsumura Y, Kore-eda S *et al*. Cutaneous infection due to *Mycobacterium interjectum* in an immunosuppressed patient with microscopic polyangiitis. *Br J Dermatol* 2008; 159 (6): 1382-4.
5. Emler S, Rochat T, Rohner P *et al*. Chronic destructive lung disease associated with a novel mycobacterium. *Am J Respir Crit Care Med* 1994; 150 (1): 261-5.

CASE REPORT

JIACM 2010; 11(1) : 72-77

Diagnostic Challenge and Pitfalls of3 Tumefactive Multiple Sclerosis

Geeta A Khwaja, Rajeev Ranjan**, Meena Gupta***, Debashish Chowdhury*, Om Prakash***

Abstract

Tumefactive multiple sclerosis (TMS) represent one type of a spectrum of CNS inflammatory demyelinating disease with atypical clinical and radiographic features that are often misdiagnosed as a neoplastic or infective process. This case report highlights the diagnostic pitfalls and provides a brief account of TMS.

Introduction

Multiple sclerosis (MS) represents only one member of a family of closely related CNS inflammatory demyelinating disease (IDD). The clinical and imaging features of prototypic MS are well-defined, but the atypical clinical and radiographic features of TMS and other fulminant variants of MS such as Marburg variant and Balo's concentric sclerosis can often confound the diagnostic process as highlighted by this case report.

Case history

A 19-year-old, previously healthy female, reported to us in April 2009, with a 10-months history of neurological dysfunction. Her first symptom was a single, generalised, tonic-clonic seizure (May, 2008), followed 2 weeks later by recurrent headache and vomiting which persisted for the next one-and-a-half months. She complained of episodic, severe, non-throbbing, bifrontal headache which lasted for 10 - 15 minutes and would occur once in every 3 to 5 days or several times a day, and this was often accompanied by vomiting. There was no history of fever or any other associated complaints. Two weeks after the onset of these symptoms, the patient was subjected to a MRI brain scan (18/6/08) which revealed large predominantly white matter hyperintensites in the left frontal and right temporal region with mass effect on T2 weighted and FLAIR images (Fig. 1) and hypointense areas with irregular open ring enhancement in the same areas in sagittal T1 weighted contrast images (Fig. 2: A and B). She was diagnosed as a case of multiple intracranial tuberculomas/abscess and prescribed four drug ATT (Rifampicin + INH + Pyrazinamide + Ethambutol) along with prednisolone (40 mg/day) and phenytoin (200 mg/day) by the treating physician in June 2008. Her headache and vomiting abated within one month of initiating ATT and she remained asymptomatic for the next 2 months. Four months post-illness onset however, she developed a cognitive impairment in the form of forgetfulness for recent events along with a difficulty in wearing clothes (tendency to wear the shirt wrong way or inside out). There was, however, no history of hallucinations, delusions, or any change in behaviour

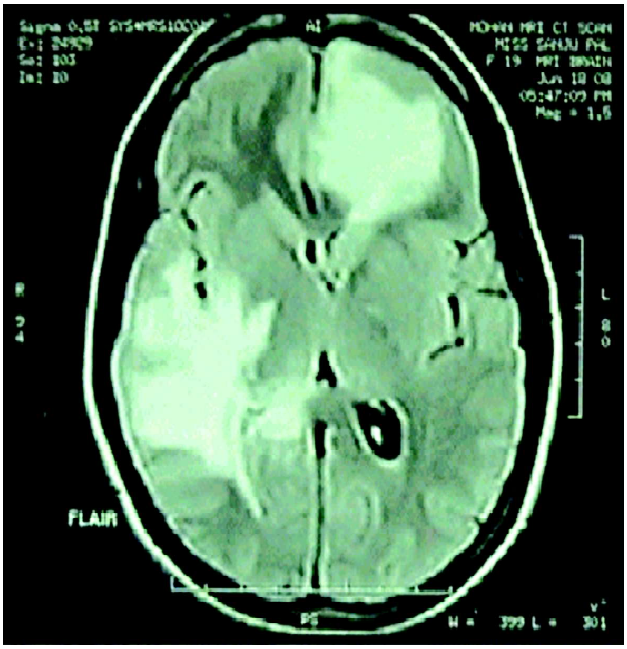
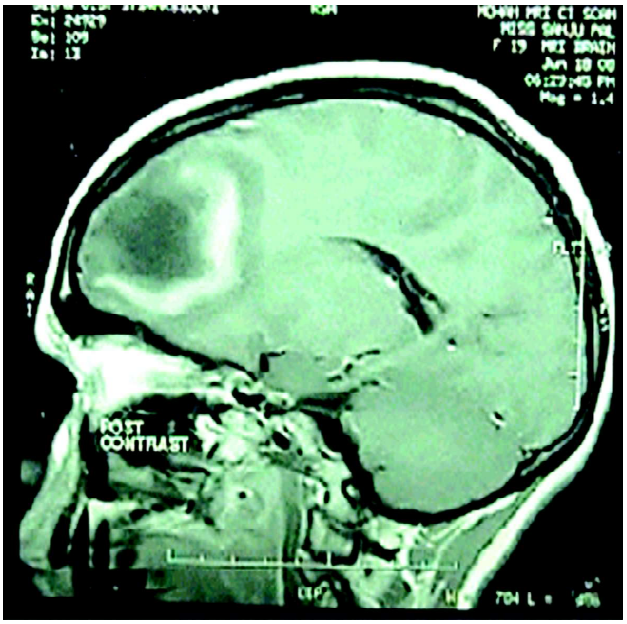


Fig. 1: *Initial MRI (18/6/08) - Axial FLAIR images showing large predominantly white matter hyperintensites in the left frontal and right temporal region with mass effect.*

** Professor, ** Senior Resident, *** Director-Professor and Head, Department of Neurology, G. B. Pant Hospital, New Delhi - 110 002.*



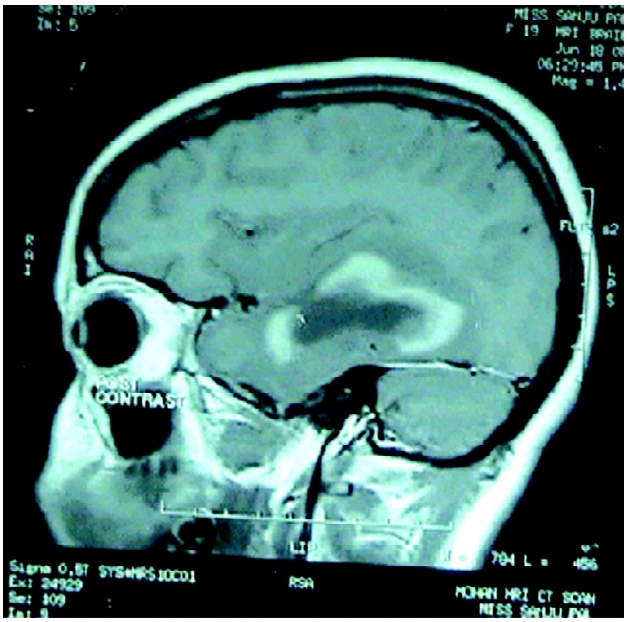


Fig. 2A and B: *Initial MRI (18/06/08) – Sagittal T1 weighted contrast images showing left frontal (A) and right temporal (B) hypointense areas with irregular open ring (towards cortex) contrast enhancement.*

or personality. One month after the onset of these symptoms she developed recurrent generalised tonic seizures (4 – 5/day) which lasted for about one month and abated after stepping-up the dose of Eptoin to 300 mg/day. Subsequently (6 months post-illness onset) she developed a bilateral painless visual impairment with difficulty in reading, which however, recovered spontaneously within 2 – 3 weeks. In December 2008 (7 months post-illness onset) she developed trunkal and gait ataxia with difficulty in sitting and walking in the absence of any limb weakness or loss of sensation. One month later (January 2009), she developed acute onset weakness of the left lower limb (power grade zero), followed 1 week later by similar weakness in the right lower limb and 3 to 4 days later by mild weakness of both the upper limbs and trunk without any sensory or bladder complaints. A follow-up contrast CT scan head (16/01/09) revealed significant resolution of the earlier lesions with new bilateral, parasagittal ring-enhancing lesions and surrounding oedema (Fig. 3). ATT was continued by the treating physician. She however, remained bed bound for the next one month, but subsequently started improving and was walking without support a month later, even though she did not recover fully. There was no history of fever or any other systemic complaints at any point in the course of the illness. Compliance for ATT was good, and besides phenytoin she had also received prednisolone in varying doses of 10 – 20 mg/day off and on for the last 10 months. There was a past history of taking a one year ATT course for TB of the right knee joint, 6 years back.

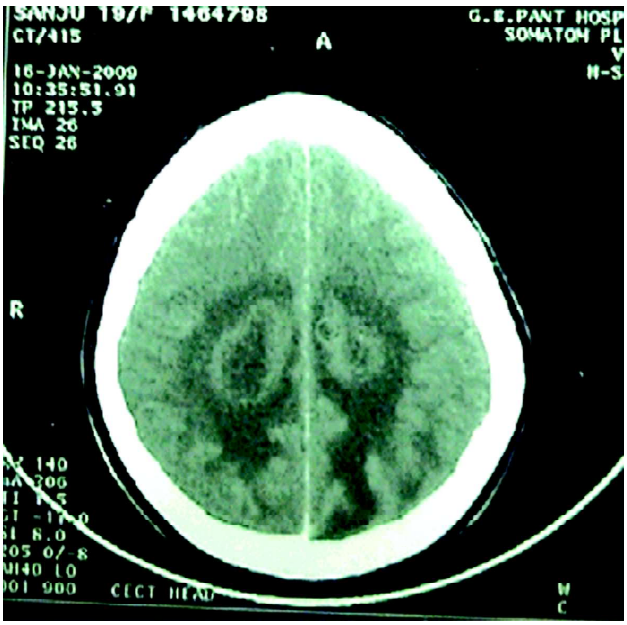


Fig. 3: *Follow-up CT scan head (16/01/09) – Post-contrast axial image showing bilateral, large, parasagittal ring-enhancing lesions with surrounding oedema (with significant resolution of the earlier lesions).*

On examination at the time of presentation to us (April 2009), the patient was fully conscious and alert. General physical, cardiovascular, respiratory and abdominal examination was normal. Mini Mental State Examination (MMSE) score was 23/30. On detailed higher mental function examination, language function was found to be preserved. Positive findings included a lack of orientation in time, mild memory impairment, calculation difficulty, dressing and constructional apraxia, suggestive of right-sided parietal dysfunction (Fig. 4). Cranial nerves including fundoscopy were normal except for left sided hemianopia. There was no nystagmus or dysarthria. On motor examination, power was normal in all the 4 limbs but there was a generalised hyper-reflexia (left > right) with increased tone in both the lower limbs and an extensor plantar response on the left side suggestive of pyramidal involvement. Sensations were intact. Upper limb coordination was preserved, but the patient displayed a significant heel-shin incoordination with gait ataxia suggestive of a bilateral cerebellar dysfunction.

In view of a 10-months history of a relapsing, remitting neurological illness characterised by 5 distinct poly-symptomatic attacks separated by intervals of > one month and a significant resolution of the initial large necrotising ring-enhancing lesions and appearance of fresh lesions at different sites, a diagnosis of TMS was entertained and ATT stopped.

Routine investigation including haemogram, blood sugar, KFT, LFT, lipid profile, serum lactate, ECG, and X-ray chest were all within normal limits. ELISA for HIV I and II and connective tissue profile including rheumatoid factor and ANA was negative. A follow-up MRI (17/04/09), revealed bilateral large parasagittal white matter hyper-intensities on axial T2 weighted image with two small ring-enhancing lesions in the same area on the T1 weighted post contrast scan (Fig. 5 A and B). Coronal FLAIR image revealed bifrontal white matter hyper-intensities extending across the corpus callosum (Fig. 6). There was a significant resolution of the initial MRI lesions. MR spectroscopy (MRS) of the lesion revealed increased choline and lipid peaks and decreased NAA peak suggesting either a neoplastic or demyelinating pathology. Evoked potentials studies revealed bilateral prolonged VEPs (Right-115 ms; Left-108 ms) with normal BAER and SSEP responses. On CSF analysis, cell count was 8 cells/cmm, sugar level 54 mg/dl, protein 46 mg/dl and oligoclonal bands were absent.

Discussion

MS is an autoimmune inflammatory demyelinating white matter disease of the CNS, that is diagnosed by demonstrating clinical and/or radiographic evidence of dissemination of the disease in time and space[1]. Typical symptoms and signs of MS include sensory syndromes, hemiplegia, paraplegia, and optic neuritis. Ataxia, bladder symptoms and cognitive dysfunction may also be

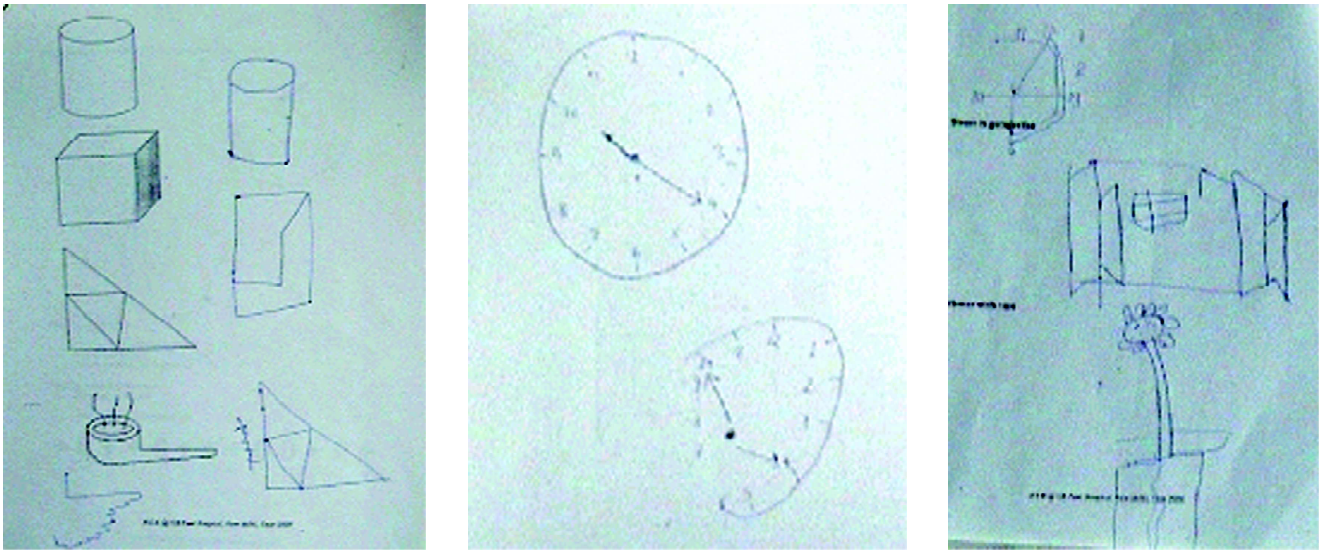


Fig. 4: *Constructional apraxia with left-sided neglect.*

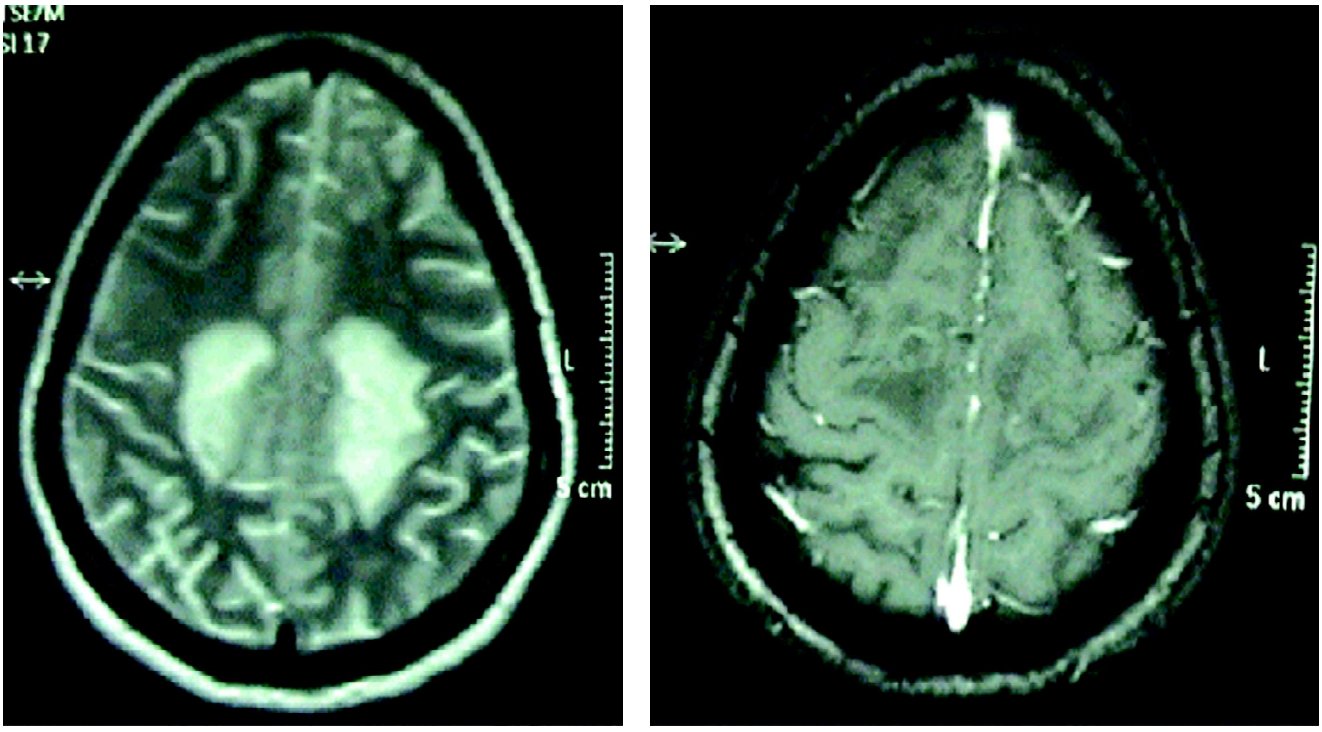


Fig. 5A and B: *Follow-up MRI (17/04/09) – Axial T2 weighted image (A) showing B/L parasagittal white matter hyper-intensities and T1 weighted contrast image (B) showing two small ring-enhancing lesions in the same area.*

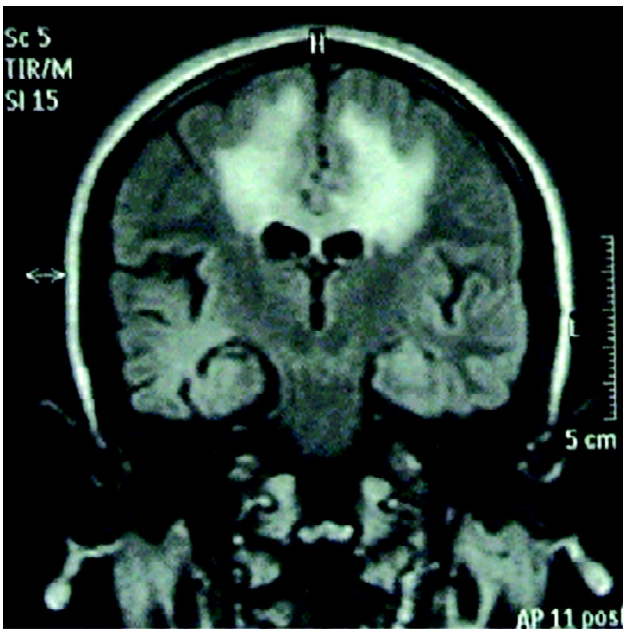


Fig. 6: *Follow-up MRI (17/04/09) – Coronal FLAIR image showing bifrontal white matter hyper-intensities extending across the corpus callosum.*

encountered. A relapsing-remitting course is seen in over 80% cases[2]. TMS however, has an atypical clinical and radiographic profile with at times a fulminant course that may pose a diagnostic dilemma. In our case, the initial presentation with a GTCS followed by recurrent episodes of headache and vomiting and the MRI findings of two large multifocal ring-enhancing lesions with mass effect led to the erroneous diagnosis of tubercular abscess. Abatement of the clinical symptoms within a month of initiating ATT was taken as evidence of response to the therapy. The subsequent relapsing and remitting, polysymptomatic course of the illness with 5 distinct attacks over a 10-months course and the changing MRI picture with significant resolution of the initial space occupying lesions and appearance of fresh lesions at different sites however suggested the possibility of a demyelinating pathology or TMS. Prolonged VEPs in both the eyes further supported the diagnosis.

TMS – like prototypic MS – is most common in the 15 – 40 years age group[2]. The female: male ratio is 1:1 as compared to 2:1 for prototypic MS[2]. The clinical course may be characterised by a single event or a relapsing remitting course[3]. The onset may be with unifocal disease but most cases have a multifocal disease at onset and develop a RRMS on follow-up, as was the case with our patient[3].

Clinical features of TMS may be indistinguishable from a brain tumour and include headache and altered mental status due to raised intracranial pressure and cerebral oedema associated with the large unifocal or multifocal space occupying lesions. Our patient also presented with seizures, headache, and vomiting. Seizures have been reported in up to 1 to 3% cases of MS and 6% cases of TMS[3,4]. Cognitive deficits including memory loss, mental confusion, and impaired attention are often overlooked, but observed in upto 20% cases of MS and reflect early grey matter cortical involvement[5]. Other cortical symptoms like aphasia, agnosia, and apraxia are rare but can occur. A history of forgetfulness, dressing and construction apraxia was observed in our case also. Aphasia has been reported in up to 1% cases of MS and 17% cases of TMS[3]. Symptomatic visual field defects due to involvement of the posterior visual pathways have been reported in up to 10% cases of TMS[3]. A left homonymous hemianopic field defect was observed in our case also. Systemic symptoms such as fever, may accompany the focal, multifocal or non-localising neurological deficits, raising the possibility of an infectious and/or inflammatory disorder including abscess, vasculitis, or granulomatous disease. Since misdiagnosis can result in unwarranted procedures and treatment, it is critical for the neurologist to be aware of this diagnostic pitfall. Focus on historical details and the relapsing-remitting course of the illness offered important diagnostic clues with regards to the demyelinating nature of the illness in our case.

CSF analysis in TMS may reveal a pleocytosis of up to 50 cells/cmm, with a normal sugar level and elevated protein in upto 75% cases[2]. CSF oligoclonal bands (OCB) may be seen upto 33 to 75% cases[3]. Some studies have suggested that absence of OCB in the CSF may be associated with a relatively benign course, with delayed time to a second event and disability progression, but in our case this pattern was not observed[3].

MRI findings in TMS may include a single or multiple large space occupying lesions with associated oedema and mass effect. In contrast to prototypic MS where the lesions are 3 to 16 mm in size, the demyelinating lesions of TMS are usually > 2 cms in size in 80% cases[3]. The enhancement pattern of tumefactive lesions is more often the open ring type, the incomplete portion of the ring abutting the cortical grey matter or basal ganglia, as was observed in our case[3,6]. Although the ring associated with abscesses and neoplasms in more often complete, a closed ring may also be observed with IDD. Moreover, a variety of enhancement patterns including heterogenous, homogenous, open ring, multiple closed rings, cotton ball, nodular, punctuate, concentric, patchy and diffuse have also been reported in CNS IDD, as have smaller lesions, as was seen in the follow-up MRI of our patient[3]. Some series suggest that the lesions are more common in the subcortical as compared to the periventricular white matter and are more often supratentorial. Topography-wise, the lesions tend to be juxtracortical, and may extend into the cortex accounting for the cortical manifestations[2]. On occasion, the lesions may spread across the corpus callosum in a butterfly configuration, as was observed in our case, simulating an infiltrative astrocytoma or lymphoma[3,7]. In one large series of 168 cases of TMS, this pattern was observed in 7% cases, multiple lesions in 83% and unifocal lesions in 17% cases only[3]. Advanced physiological MRI with magnetisation transfer, diffusion MRI, perfusion MRI and proton MRS may allow for noninvasive measurement of certain biochemical changes associated with these lesions, allowing distinction from neoplastic tumour, but are often inconclusive as was the case with our patient.

Reasons for brain biopsy in CNS IDD usually include atypical clinical presentation (e.g., encephalopathy, seizures, aphasia), older age, and large enhancing MRI lesions with associated oedema and mass effect and the limited ability of the MRI to differentiate inflammation from tumour. Pathological findings in TMS typically include hypercellular lesions with confluent demyelination, abundant foamy macrophages containing myelin debris, reactive astrogliosis, 'relative' axonal preservation, and variable perivascular and parenchymal lymphocytic inflammation[3]. Atypical astrocytes with fragmented nuclear inclusions (Creutzfeldt Peters cells) in the MS lesion can be confused with tumour cells[2,3]. However, these cells are rather evenly distributed in the MS lesions, and not clumped together as is common in gliomas. A misdiagnosis of low grade astrocytoma has been reported in upto 30% case of TMS[3]. Biopsy, however, was not performed in our case.

The first attack TMS may be difficult to distinguish from other MS variants. The Marburg variant is a monophasic, rapidly progressive and aggressive form of MS with widespread, multiple large confluent areas of demyelination in the cerebral hemispheres or the brainstem[2]. The clinical course is fulminant with signs of meningism, headache, altered conciousness, seizures, raised ICP and death occurring within months to one year. On pathologic examination, the lesions are more destructive with severe axonal injury, immunoglobulin (IGg) deposition, and complement activation at the lesion sites[2]. Because of similar clinical, laboratory, and radiologic findings and monophasic course, the Marburg variant of MS may be difficult to distinguish from ADEM which however, has a better prognosis. The clinical and radiodiagnosis profile of Balo's concentric sclerosis is similar to Margburg variant but pathologically the lesions are characterised by alternating concentric layers of myelinated and demyelinated tissue (may be seen as alternating hypointense and hyperintense rings on T2 weighted MRI scans)[2]. The other hallmark is a selective myelin associated glycoprotein (MAG) loss and oligodendrocyte apoptosis[2]. The clinical as well as the pathological, diagnostically challenging nature of CNS IDDs needs to be appreciated. A good neurological history, CSF, and evoked potential studies may, however, obviate the need for a brain biopsy as was the case with our patient.

Earlier studies had suggested that acute episodes of TMS are usually isolated or remain monophasic. A recent large study has however shown that around 70% cases ultimately develop clinically definite MS with radiographic evidence of disease progression[3]. Though some patients of TMS may have a fulminant and fatal course, most do well despite the atypical and aggressive clinical presentation at disease onset, and prognosis is not greatly affected by the appearance of tumefactive lesions. In one study, the median time to the second 'MS defining' clinical episode was 4.8 years for TMS, as compared to 1.9 years for prototypic MS[3,8]. Our patient however, had 5 relapses within a 10 months span.

Most patients experience symptomatic improvement and reduction or disappearance of radiographic abnormalities after steroid therapy. Our patient was discharged on a low maintenance dose of 10 mg of prednisolone per day. Plasma exchange should be considered in patients who fail to respond to steroids.

In conclusion, an increased awareness of the broad spectrum of the closely related CNS inflammatory demyelinating diseases and their atypical clinical and radiographic features is important for arriving at the correct diagnosis. Since misdiagnosis can result in unwarranted procedures and treatments, it is critical for the neurologist to be aware of this diagnostic pitfall. History of a typical relapsing and remitting illness course, with dissemination of lesions in time and space may obviate the need for a brain biopsy even in cases with large unifocal or multifocal enhancing mass lesions in the CNS, if the diagnosis of TMS is kept in mind.

References

1. Mc Donald WI, Compston A, Edan G *et al*. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the Diagnosis of multiple sclerosis. *Ann Neurol* 2001; 50: 121-7.
2. Pittock SJ, Lucchinetti CF. The pathology of MS. New insights and potential clinical applications. *The Neurologist* 2007; 13: 45-56.
3. Lucchinetti CF, Gavrilova RH, Metz I *et al*. Clinical and radiographic spectrum of pathologically confirmed tumefactive multiple sclerosis. *Brain* 2008; 131: 1759-75.
4. Nyquist PA, Cascino GD, McClelland RL *et al*. Incidence of seizures in patients with multiple sclerosis: a population-based study. *Mayo Clin Proc* 2002; 77: 910-2.
5. Hauser SL, Goodkin DE. Multiple sclerosis and other demyelinating disease. Harisons Principles of Internal Medicine. Editors: Braunwald E, Fauci AS, Kasper DL *et al*. Jameson. Publishers – Mc Graw-Hill. 15th edition , 2001; Pg. 2452-61.
6. Rolak LA, Fleming JO. The differential diagnosis of multiple sclerosis. *The Neurologist* 2007; 13: 57-72.
7. Rieth K, Di Chiro G, Cromwell L. Primary demyelinating disease simulating glioma of the corpus callosum. Report of three cases. *Neurosurgery* 1981; 55: 620-4.
8. Confavreux C, Vukusic S. Natural history of multiple sclerosis: implications for counselling and therapy. *Curr Opin Neurol* 2002; 15: 257-66.

INSTRUCTIONS TO AUTHORS

General information

1. Send all articles in triplicate (with a floppy 3½", wherever possible) by registered post/courier. Please label the floppy with file name & programme on which it is made, e.g., MS-Word, Word Perfect, Pagemaker.
2. All articles must be accompanied by a covering letter signed by all authors confirming that the article has not been published or submitted elsewhere, either in part or in whole. This restriction does not apply to abstracts published in connection with scientific meetings.
3. The editorial board may modify the article partially or wholly to bring it to the set pattern of the journal to ensure continuity in style.
4. The views expressed in an article will remain the opinion of the author(s), and the editorial board accepts no responsibility for the same.
5. The papers which are published in *JIACM* become the property of the *Journal* and should not be published or reproduced in full or in part elsewhere without the permission of the Editor.

Original articles

- Should be typed double-space on A4 size bond paper with a 3 cm margin all around. Type on one side of paper only.
- Articles should not exceed 10-12 typewritten pages, including references, but excluding tables.
- The sequence of an article should be as follows :
  - Title page
  - Abstract
  - Keywords
  - Introduction
  - Material & Methods
  - Results
  - Discussion
  - Acknowledgment
  - References
  - Tables
  - Legends to figure(s)

A. Titles and Author(s) name

- On a separate page.
- Concise & descriptive title.
- Author(s) name be given as initials followed by surname.
- The academic degree and designations with name of institution be given at bottom of page after superscribing individual authors names with asterisks.
- Give address (postal as well as e-mail) for communication at bottom of page alongwith telephone and fax numbers.

B. Abstract

- This should be given on a separate page.
- Should not exceed 250 words for original article and 100 words for a case report, and should contain 3-10 keywords at conclusion of abstract.
- Should include background, aims of study, methodology, results, and conclusion. The problem(s) being addressed in the study, and conclusion of its result, must be expressed clearly in the abstract.

Review Articles and Update Articles should also be preferably sent alongwith an abstract.

C. Key words

- These are required for indexing of the article and the *Journal*.
- The key words should not include words in title as title is automatically indexed.
- The key words may be taken from Medical Subjects Headings (Me SH) published under these headings.
- Discussion should avoid repetition of results or review of literature.
- Text should not contain data given in tables.
- Abbreviations must be avoided in title, subtitle, and summary.
- Only the generic name of a drug should be used. Brand names may be used in material & methods section only when these have been used in research.
- Materials taken from other sources must be accompanied by a written statement from the author and the publisher giving permission to the *Journal* for reproduction of the same.

D. Statistical methods

- Describe methods used, in detail.
- Present the results in a logical sequence in the text, tables, and illustrations.
- In discussion, emphasise newer aspects of the study and the conclusions that follow from them.

E. Tables

- Type each table double-space on a separate page.
- Provide a title for each table, and indicate its position in the text.
- Number the tables in Arabic numerals.

- Put explanatory note in the footnote (and not in the heading).
- Do not use internal horizontal and vertical rules.
- Number of tables should be in proportion to length of the text.

F Illustrations

- As far as possible, the figures should be professionally designed, on glossy paper, and large enough to be legible even after reduction, so as to fit the width of a single column.
- The back of each figure should have the name of author, sequence number, and proper orientation (e.g., 'top') marked in pencil.
- Figures should be unmounted.
- Photographs and illustrations should be 7x7x7 cm or 14 cm.
- Electron micrographs should have internal scale markers.
- Photographs of patient(s) should be accompanied by permission, or the subject should not be identifiable.
- Legends for illustrations should be triple-spaced on a separate paper.
- Colour prints are encouraged. Send both transparencies and glossy prints for this purpose.
- **Abbreviations.** If a term appears repeatedly, an abbreviation may be introduced in parentheses at the initial mention of the term and used thereafter in place of the term.
- **References to drugs.** The generic name of a drug should be preferred as a general rule. However, the full name, or the commercial name of the drug, as well as the name and location of the supplier, may be given if appropriate.
- **Copyright.** Material that has appeared elsewhere must be submitted with a legal release from the holder of the copyright.
- **Ethical consideration.** Reports on biomedical research involving human subjects must include a statement that the informed consent was obtained from each subject, or from his/her guardian. Reports on human experimentation must include a statement of approval by a local Human Investigations Committee, in accordance with the precepts established by the Helsinki Declaration.
- **Conflict of interest.** The author is required to disclose any commercial or other association that may constitute a conflict of interest in connection with the submitted manuscript.

G. References

1. The Vancouver system of references should be used.
2. References in text should be given as a number within brackets (rather than superscript figures).
3. The number of references should not exceed 30 for an original article, 10 for a short article, 8 for a case report, and 3 for a letter to the editor.
4. References should be numbered in order of their appearance in the text and not alphabetically.
5. Personal communications are to be identified in the text and not in the reference.
6. Cross references ("Quoted by _____") should be avoided.
7. As far as possible, Indian references should be preferred.
8. Names of all authors (upto four) should be mentioned; if there are more than four, then the first three names are mentioned followed by '*et al*'.
9. The author is wholly responsible for accuracy and completeness of the references.
10. As far as possible, only recent references should be included.

The form of references is as follows:

From a Journal

Name of Author(s). Title of the Article. Name of Journal Year; Vol: Page No(s).

From a chapter in a book

Name of Author. Name of Topic. Name of Editors. Name of Book. Place of Publication. Name of Publishers, Year; Vol: Page(s).

From a book

Name of Author or Editor. Name of Book. Place of Publication. Name of Publisher, Year; Vol: Page(s).

Reference to Personal Communications and to unpublished material must be incorporated, in parentheses, at the appropriate place in the text.

References to Congress abstracts should be cited in the reference section if they have been published previously in an official book of abstracts from the Congress.

H. Correspondence

Letter to the editor may comment on articles published in the journal, or may provide additional original data or information. The text should not exceed 500 words and a maximum of 3 references are permitted. The letter must be received within six weeks of the article's publication.

I Review and actions

The editorial board examines the manuscripts and sends them to reviewers. The author(s) of an article not accepted for publication will be informed within six weeks usually, but decision on potentially acceptable articles may take longer.

FORMAT FOR NOMINATION FOR ORATION

(Eight copies of format and eight copies each of important publications to be sent)

1. Name (in full) : .....
2. Designation : .....
3. Address : .....

   ..... Pin Code : .....

   Phone : ..... Fax : .....

   E-mail : .....
4. Oration for which nominated : .....
   (A person can be nominated for more than one oration simultaneously but can be awarded only one oration in his life time)
5. Field of interest and subject/title of oration, if awarded : .....
6. Bio-data and Justification for Nomination : .....
   (Please use separate sheets)

Signature ..... (Proposer)
Name:
Fellowship/Membership No.:

Signature ..... (Seconder)
Name:
Fellowship/Membership No.:

CONSENT OF NOMINEE

I hereby agree for the above nomination and undertake to deliver the oration if awarded at the appointed day and time. I have not been awarded any oration by the "Indian Association of Clinical Medicine" earlier.

Signature

(Name of Nominee)

Fellowship/Membership No. .....

RULES FOR ORATION/ORATORS:

1. No TA/DA shall be provided to the orator.
2. It is obligatory and compulsory for the awardee of oration to deliver it at the appointed day and time during the annual conference. No alternations shall be possible.
3. Oration if not delivered shall lapse and shall not be carried forward ordinarily.
4. A person can be nominated for any or all the orations and shall be considered for the orations he has been nominated for. However, only one oration can be awarded.