C O N T E N T S

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VIEWPOINT

JIACM 2011; 12(4): 260-2

Integrated medical care system (Complementary systems of medicine – Are they scientific?)

*BM Hegde**

"The only thing worse than being talked about is not being talked about".

– Oscar Wilde (1854-1900).

"Science is making models, mostly mathematical constructs, which, with verbal jargon, are supposed to work", wrote John von Newmann, a Hungarian born American scientist! With this definition, modern medicine – as it is practised today – becomes most unscientific. The human body follows the holistic non-linear mathematical model in its functioning, while the modern medical model uses the linear mathematical rule of Newtonian physics with its faulty deterministic predictability model – a square plug in a round slot. Even then people claim that modern medicine is scientific. In truth, the science of modern medicine is only a statistical science and not true science. Even the Institute of Medicine (IOM), the highest body appointed by the Academy of American Science to oversee and audit the medical field, has now accepted the definition of medical care as *Whole Person Healing* (WPH) in place of the present reductionist model of organ based sub-speciality quick fix mending, in their February 2010 meeting at Washington DC.

Douglas C Wallace, a noted American professor of genetics, in his classic, *Mitochondria as Chi*, in the journal *Genetics* (2008; 179: 727-35) has shown that all reductionist chemical molecules used in our therapeutics, being dextrorotatory while the body molecules are levorotatory, destroy body cells. Whereas all the Eastern herbal drugs are accepted by the body as food (they are also levorotatory) and help the system! My former teacher at Harvard – a Nobel Laureate – cardiologist, Bernard Lown, wrote recently in a letter to the New Yorker, along with his junior colleague, Graboys, thus: "We believe the modern medical model has become increasingly reductionist: human beings are seen as repositories of malfunctioning organs that need repair. This view results in an onslaught of tests and uncertainty. Doctors often take refuge behind technology because it is easier and less time-consuming than talking with a complex human being who is their patient". (*The New Yorker* 5/17/99).

Modern medicine has become a costly chaos with no end in sight. We now have significant problems that beg urgent solutions. As Albert Einstein once observed: "The significant problems we have cannot be solved at the same level of thinking with which we created them". We obviously need a new approach in medical science to solve our health problems. The solution to the present human-made and drug-industry-protected health problems of society can only be physiology-based. Integrated systems of medical care, where the scientifically proven safe methods of treatment from other complementary systems should form the basis of future medical care system alongwith corrective and trauma surgery from modern medicine; the latter together have saved many lives. One look at the common coronary bypass surgery from one of the best brains in cardiology, Bernard Lown, tells it all: "Our experience and research, and those of others, demonstrate that a very significant percentage of patients undergoing bypass surgery – perhaps as many as two-thirds – can safely defer or altogether forego these procedures by managing their heart problems with medication. Regrettably, much of the rush to invasive procedures is driven by non-medical factors – principally economic ones".

"Perhaps the most valuable result of all education is the ability to make yourself do the thing you have to do,

* *Padma Bhushan; Former Vice-Chancellor, Manipal University;*
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when it ought to be done, whether you like it or not; it is the first lesson that ought to be learned; and however early a man's training begins, it is probably the last lesson that he learns thoroughly". Wrote Thomas H. Huxley while philosophising on human ethical values. Medical ethics is nothing but good human ethics. The recent Northwick Park tragedy is still fresh in our mind about the fallacy of extrapolating animal data to humans, while the tragedy of similar data in the case of milrinone, which worked totally differently in rats vis-à-vis humans, is all but forgotten from recent memory.

The question of complementary systems of medicine being unscientific is a figment of imagination of those that want conventional medicine to flourish for ever as it has become the biggest milch cow for the industry, by destroying anything that might endanger its supremacy. Exhaustive studies of all the "statistical science" of modern medicine shows that most of it cannot stand the test of strict validation as shown by David Eddy using his new computer model ARCHIMEDES, at least in chronic illnesses to begin with. Even in the field of emergency care, where modern medicine seems to be a blessing, there are large gaps in our understanding of the management strategies! Outcome audits of such use in Vietnam and Falklands wars leave much to be desired in emergency grievous trauma care.

Many complementary systems that are being scientifically studied by the Whole Person Healing Group of scientists based in Washington DC[1] have shown the existence of vast amounts of observational research going back thousands of years in some systems like Ayurveda. It will be easy to authenticate these data using the modern "scientific" methods to bring the best in those systems to main line medicine. The data base available could shorten the drug invention and could totally eliminate our "wrong" method of extrapolating animal data to humans. In addition, we have also been using reductionist science to study a chaotic non-linear dynamic human system, wherein arrhythmia could be healthy while rhythmia could be illness!

Just as quantum physics upset the Newtonian laws of deterministic predictability, complementary systems might upset modern medical foundations. Newtonian laws should result in the electrons destroying their own nucleus at the atom. Electrons also do not follow the electromagnetic forces at that level! Medical science has to learn a lot from quantum mechanics but, that would need a quantum leap in the thinking of our established "leaders" in the monetary economy. Ayurveda proclaims that the "well" should be preserved and only the "ill" should be treated (leave the "well" alone wrote William Osler) as there is no way to predict the future of a dynamic organism like the human body using phenotypic data alone! Ayurveda also shows how, at the quantum level, energy and matter have no difference – most advanced quantum physics! ("Matter is not made up of matter". – Hans Peter Durr).

Homoeopathy has been found to have a sound scientific base in that all homoeopathic medicines are either in the nano or piko forms; consequently, these cannot be detected by the conventional chemical analysis. Since they are in nano forms they are obviously safe to the human system. The bench mark of modern medical research, the Randomised Controlled Trials (RCTs), have now been torn into bits as unscientific by many studies. "Emblematic of the later history of clinical trials is the fact that the streptomycin study, extolled then and since as a breakthrough in medicine (first ever RCT), in fact yielded disappointing results: the treated cases showed improvement only for three months and thereafter began to deteriorate (MRC 782)".

The new integrated system should only concentrate on symptomatic patients. There are no silent killers in human illness scenario. The screening for occult diseases is a new trick of the trade in modern medicine for economic reasons! This applies especially to cancer which is being increasingly detected at its pre-symptomatic stage these days. The five-year relative survival rate is thus improved! But the lives of the patients are not extended by even ONE day. Nothing new has been done to affect the course of the disease, and although the patient is not living longer, it appears that there is improved five-year survival if one measures the survival from the date of diagnosis. Early diagnosis only makes the life more difficult to live with fear and the unpleasant side-effects of cancer treatment methods in vogue these days!

Efforts are on in some centres to authenticate healing

methods in many other complementary systems of medicine scientifically to be included in the new integrated WPH system. It is also mandatory to abandon the "disease care" model of today which has far outlived its usefulness in favour of preserving the wellness of human beings. Thinkers even in the West have felt that the disease era, with its labelling human beings, has come to an end. Writing an article, "End of Disease era", Mary Tinnetti and her colleague T. Fried from Yale University had this to say: "The time has come to abandon disease as the focus of medical care. The changed spectrum of health, the complex interplay of biological and non-biological factors, the ageing population, and the inter-individual variability in health priorities render medical care that is centred on the diagnosis and treatment of individual diseases at best out of date and at worst harmful. A primary focus on disease may inadvertently lead to under-treatment, overtreatment, or mistreatment".

Integrated system with new classification of illnesses, suggested by me, years ago, should be the future. To cap it, doctors must become human and humane in their approach to patients in distress as their role in medical care delivery is more potent than all the medicines put together. The placebo doctor can provoke the human immune system much more powerfully than all medicines put together. This has now been shown to be very scientific. The forebrain secretes powerful chemicals with a good placebo response. The latter can now be studied using fMRI (functional MRI) and also blocking the chemical release with Naloxone injections (for research only). May the future re-invent modern medicine as the best integrated system of human illness care.

"Men stumble over the truth from time to time, but most pick themselves up and hurry off as if nothing happened".

-Winston Churchill.

FLAVEDON

ORIGINAL ARTICLE

JIACM 2011; 12(4) : 263-5

HbE variants – Retrospective analysis in a tertiary care centre

S Aggarwal, S Saluja**, S Bhasin***, M Sharma****, DK Gupta*****, B Gupta******, V Mittal****

Abstract

HbE disease is prevalent in the north-eastern part of India but more cases are now being reported from North India as well due to migration of population. A retrospective clinical and haematological evaluation of 60 patients of HbE variants attending haematology OPD of our hospital in the last seven years was carried-out. Clinicians of North India should consider it in the differential diagnosis of anaemia with or without splenomegaly for accurate diagnosis and management.

Key word: HbE.

Introduction

HbE (Haemoglobin E) is the second most prevalent haemoglobinopathy after HbS[1], showing the highest prevalence in South-East Asia[2]. In India, it is prevalent in the north-eastern states[3]. This retrospective study highlights the clinical and haematological profile of HbE variants presenting for the first time in our haematology department from January 2000 to December 2007, and emphasises the consideration of HbE disease in the differential diagnosis of microcytic hypochromic anaemia with or without splenomegaly.

Material and methods

Our study is a retrospective analysis of diagnosed cases of HbE disease. All data were analysed for detailed clinical history, examination. It included history of ethnic origin, age, sex, clinical symptoms like fatigue, weakness, jaundice, dyspnoea, history of blood transfusion and fever, and signs of anaemia, jaundice, and organomegaly. Family history was recorded wherever possible. All patients were investigated with complete haemogram, reticulocyte count, pheripheral smear, liver function test, iron studies, Hb electrophoresis (Agarose gel at alkaline pH 8.6) and HPLC (high performance liquid chromatograhy). HbF was quantitated by alkali denaturation methods also.

Diagnosis was based on electrophoretic pattern of Hb as HbE trait (heterozygous HbE), HbE disease (homozygous HbE) or doubly heterozygous disease (HbE-β thalassaemia). Patient showing bands at HbA_2/E position with increased HbE level as HbE disease; bands at HbA/A_2/E with HbE (20 – 45%) as HbE trait, and bands at HbE/F positions with increased HbF (5.5 – 85.4%) as doubly heterozygous HbE-β thalassaemia was diagnosed accordingly. Skull radiographs and bone marrow aspiration were done in doubly heterozygous HbE-β thalassaemia patients.

Results

Based on electrophoresis and HPLC, 10 patients were diagnosed as homozygous (HbE disease), 16 as heterozygous (HbE trait) and 34 as HbE-β thalassaemia. Mean age of presentation was 15 years in HbE disease (range 2 – 40 years), 13.2 years (range 5 – 30) in HbE trait and 4.42 years (range 2 – 20) in HbE-β thalassaemia respectively. Out of 60 patients, 49 patients presented with variable signs and symptoms of anaemia (35 in HbE-β thalassaemia, 8 in HbE disease and 6 in HbE trait). Mean value of haemoglobin was 7.8% in homozygous, 11.1% in heterozygous and 5.9% in HbE-β thalassaemia. In HbE trait, three came to us during investigations for PUO, 12 with refractory anaemia, and one patient was a follow-up of CML with persistent low haemoglobin. Iron profile was normal in all patients. Reticulocyte counts were consistently normal in HbE trait, normal to increased (2 – 3%) in HbE disease, and increased (5 – 6%) in HbE-β thalassaemia disease. Other findings observed were mild

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icterus, mild splenomegaly (3 – 5 cm), elevated bilirubin (1.2 – 2.0 mg%) and microcytic hypochromic picture with anisocytosis and target cells in HbE disease, no jaundice and splenomegaly but microcytic hypochormic picture with anisopoikilocytosis in HbE trait and moderate-to-severe anaemia, moderate splenomegaly (5 – 8 cm), elevated bilirubin (2 – 3 g%) and microcytic hypochromic picture with marked anisopoikilocytosis, normoblastaemia and target cells, hypercellular bone marrow, "hair-on-end" appearance in 4 patients of HbE-β thalassaemia. On HPLC, homozygous patients had HbE 96.6% and 3.9% HbF, heterozygous patients had HbE 28.5%, HbA 69.9% and 1.6% HbF and HbE-β thalassaemia had HbE 29.40%, HbA 42.37% and HbF 28.33%.

Discussion

In 1954, Minnich and co-workers reported the occurrence of a severe form of thalassaemia in 32 patients in Thailand which were later diagnosed as HbE variants[4]. HbE has 2α and 2β chain with substitution of glutamyl residue in the 26th fragment of the β-chain by lysine[5]. Geographically, it is prevalent in South-East Asia and the north-eastern states of India[1,2].

HbE variants usually manifest as homozygous (HbE disease), heterozygous HbE (HbE trait), and doubly heterozygous forms HbE-β thalassaemia[6]. Clinico-haematological features of HbE disease include slight decrease in haemoglobin level, mild jaundice, microcytic hypochromic picture with target cells, mild-to-moderate splenomegaly, normal to slightly increased HbF (< 6%) and single band at Hb/A2 on electrophoresis[6,7].

Heterozygous (HbE trait) usually are asymtomatic with normal to slightly decreased haemoglobin, microcytic hypochromic picture with mild anisopoikilocytosis and target cells, no jaundice, two bands at position HbA and HbE on electrophoresis with HbE in range of 20 – 45%[6,7].

HbE-β thalassaemia is the most common and severe form of the disease, which manifest with sign and symptoms of anaemia, microcytic hypochromic picture with target cells, and severe anisopoikilocytosis and normoblastaemia, increased indirect bilirubin, moderate-to-large splenomegaly and bands on electrophoresis at HbE/F position with increased HbF 5.5% to 85.4%[6]. Bands at HbA/E/F position suggests that the patient has inherited B+ gene while Bands at HbE/F position suggests that patient has inherited B0 gene.

Occurrence of HbE disease can be controlled by proper premarital genetic studies of the partners concerned especially in families known to have haemoglobinopathic affliction[5]. The management of HbE trait and disease includes only folic acid but in HbE-β thalassaemia, periodic blood transfusions may be required to maintain the haemoglobin level[6]. Indications of splenectomy are limited, i.e., massive splenomegaly, hypersplenism, frequent haemolytic crises, and significant increase in transfusion requirement[8].

In our study, we diagnosed 60 cases of HbE variants in the last 7 years in our department, based on Hb electrophoresis and HPLC. Out of 60 patients, 49 patients presented with variable signs and symptoms of anaemia which later diagnosed as HbE-β thalassaemia[2, 4], HbE disease[6], and HbE trait[3]. Out of 34 patients of HbE-β thalassaemia, 20 patients had severe anaemia requiring frequent blood transfusions (> 3 – 5 units/year) but none of them was referred for splenectomy. All the other patients were managed with folic acid. Tyagi *et al*[3] described clinico-haemtalogical profile of 50 patients of HbE syndrome (43 HbE-β thalassaemia, 7 HbE disease). In this study, only 2 of HbE disease were symptomatic comparable to our study which showed symptoms of mild anaemia in all patients. In 43 patients of HbE-β thalassaemia, only 13 showed requirement of blood transfusions (> 2 – 3 units/year) but in our study 20 patients out of 34 required transfusion therapy (> 4 – 5/year). Our study highlights the increasing prevalence of HbE variants in this part of India due to migration of population from the north-eastern states. In this study, we have attempted to describe the course of HbE variants and its management as studies from this part of India are limited[3].

In conclusion, we advise that clinicians, haematologists, paediatricians, and pathologists should be aware of this haemoglobinopathy due to migration in this part of India. They should consider it in the differential diagnosis of microcytic hypochromic anaemia with or without splenomegaly to avoid unnecessary prescription of iron salts. In families who are at high-risk of inheriting HbE-β

thalassaemia, genetic counselling should be done to decrease the prevalence of this disease and regular follow-up is necessary to delay transfusion therapy until patients develop anaemia symptoms so as to avoid complications associated with transfusions and chelation therapy[9].

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OLMEZEST

ORIGINAL ARTICLE

JIACM 2011; 12(4) : 266-70

Correlation of portal vein diameter and splenic size with gastro-oesophageal varices in cirrhosis of liver

Lopamudra Mandal, Sanjay Kumar Mandal**, Dipanjan Bandyopadhyay**, Saumik Datta***

Abstract

This study was conducted to find out the correlation of portal vein diameter and splenic size with gastro-oesophageal varices in diagnosed cases of cirrhosis of liver.

Eighty-two patients with cirrhosis of liver were selected for the study. Ultrasonography was performed in all cases to note the portal vein diameter and splenic size. Oesophago-gastro-duodenoscopy was done to detect presence of varices with grades.

In the study it was found that twenty patients had no varices (grade 0) and the rest sixty-two patients developed varices. Average portal vein diameter of patients without gastro-oesophageal varices was 11.545 ± 1.514 mm and of patients with varices 13.998 ± 1.123 mm. The difference was statistically significant ($p < 0.05$). Average spleen size of patients without gastro-oesophageal varices was 13.129 ± 1.102 cm and with varices 14.997 ± 1.992 cm. This variation was also statistically significant ($p < 0.05$). There was a positive correlation between grading of oesophageal varices and portal vein diameter ($r = 0.707$; $p < 0.001$) and between splenic size with oesophageal grades ($r = 0.467$; $p < 0.001$).

The study portrays that with increase in portal vein diameter and splenic size, the chance of formation of gastro-oesophageal varices also increases and a positive correlation exists. Thus, measurement of portal vein diameter and splenic size by ultrasonography is a non-invasive predictive indicator of the development of gastro-oesophageal varices in cirrhosis of liver.

Key words: Cirrhosis of liver, portal vein diameter, gastro-oesophageal varices.

Introduction

Portal hypertension is the most common complication and also one of the important causes of death in chronic liver diseases. Increased resistance to portal blood flow due to alteration of the hepatic architecture leads to dilatation of portal vein, splenomegaly, and formation of oesophageal and gastric varices, variceal haemorrhage, ascites, hypersplenism, encephalopathy, etc.

In cirrhosis, increased intrahepatic vascular resistance is thought to be located mainly in the hepatic sinusoids[1]. Recent studies have demonstrated that in addition to the increased resistance caused by the morphologic changes of chronic liver diseases, a dynamic component of increased resistance (resulting from the active contraction of vascular smooth muscle cells, myofibroblasts, and hepatic stellate cells) is also present[2].

Portal hypertension leads to dilatation of portal vein, splenomegaly, and formation of portal systemic collaterals at different sites. The portal system and the systemic venous circulation are connected at several locations[3]. Gastro-oesophageal collaterals develop from connections between short gastric and coronary veins and the oesophageal, azygos, and intercostal veins; the result is the formation of oesophageal and gastric varices. Collaterals develop in areas where anatomic connections exist between the portal venous and systemic circulation. These are vascular channels that are functionally closed in normal conditions but become dilated in portal hypertension as a consequence of increased intravascular pressure and blood flow. These gastro-oesophageal varices are responsible for the main complications of portal hypertension and massive upper GI bleeding[3].

It is a well-known fact that portal vein diameter is usually increased in cirrhosis of liver with portal hypertension, and spleen is also enlarged in size. A few previously reported studies showed that there was a definite correlation between portal vein diameter and presence of gastro-oesophageal varices. Sarwar *et al*[4] reported that patients with portal vein diameter more than 11 mm are more likely to have oesophageal varices. Another study by Dib *et al*[5] showed that oesophageal varices developed when

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the portal vein diameter exceeded 13 mm. On the other hand, Li *et al*[6] found that haemodynamics of the portal vein were unrelated to the degree of endoscopic abnormalities in cirrhosis of liver.

Oesophago-gastro-duodenoscopy is required to detect the gastro-oesophageal varices. But the procedure is invasive, painful to the patient, and is not available in all centres. Whereas portal vein diameter and splenic size can be measured by an easily available, painless, and non-invasive method like ultrasonography (USG). The study was done to find out the correlation between the portal vein diameter and splenic size with the development of gastro-oesophageal varices.

Materials and methods

Patients attending outdoor and admitted indoor in the department of Medicine, IPGME, and R and SSKM Hospital and the Liver Clinic of Medical College, Kolkata, were selected for study.

Either previously diagnosed or newly diagnosed cases with cirrhosis of liver were taken into account. The following cases with portal hypertension were excluded from the study:

1. Cirrhosis of liver with previous history of gastrointestinal bleeding.
2. Cirrhosis of liver with portal vein thrombosis.
3. Other cases with portal hypertension, i.e., non-cirrhotic portal fibrosis, Budd-Chiari syndrome, extra-hepatic portal venous obstruction.

82 diagnosed cases of cirrhosis of liver were included for the study. Salient features in the history included occupation, alcohol intake, appetite, jaundice, swelling of abdomen, disorientation, unconsciousness, etc. Patients with history of haematemesis and malena were not taken into account. A thorough general survey was done to assess pallor, cyanosis, jaundice, oedema engorged neck veins, palpable neck glands, pulse, and blood pressure. The gastrointestinal system was clinically examined with focus on the size of the spleen, liver span, ascitic fluid, fluid thrill, and presence of any venous prominences over the abdomen. The investigations like routine blood including platelet count, liver function tests (LFTs), prothrombin time including INR were recorded from reports of previously diagnosed cirrhotic patients or performed for the newly diagnosed cases. Ultrasonography was performed in all cases and diameter of portal vein in mm and spleen size in cm was recorded. Upper gastrointestinal endoscopy was done to locate the varices.

Spleen size measurement

Spleen size was measured ultrasonographically by placing the patient in supine position, using 2 – 5 MHz curvilinear transducer in the coronal plane of section posteriorly in one of the lower left intercostal spaces. The patient was examined in various degrees of inspiration to maximise the window to the spleen. The plane of section was then swept posteriorly and anteriorly to view the entire volume of spleen. The average adult spleen measures 12 cm in length. The spleen parenchyma is extremely homogeneous and it has a uniform mid-to-low echogenicity. When the spleen enlarges, it can be more echogenic. Splenomegaly commonly accompanies portal hypertension and is a noteworthy finding[7, 8]. A maximum cephalo-caudal measurement exceeding 13 cm indicates enlargement with a high degree of reliability[9].

Portal vein diameter measurement

The portal venous supply for the left lobe can be visualised using an oblique, cranially angled sub-xiphoid view (recurrent subcostal oblique projection). The main and right portal veins are best seen in a sagittal or oblique sagittal plane[10]. In normal individuals, the portal vein diameter does not exceed 13 mm in quiet respiration, measured where the portal vein crosses anterior to the IVC[11-14]. This assessment is usually conducted with ultrasound views along the long axis of the portal vein. Respiration and patient position greatly affect the size of the portal vein and its tributaries; therefore, diagnostic measurements must be standardised by examining the patient in the supine position and in a state of quiet respiration. We followed the above method to measure portal vein diameter.

Upper gastrointestinal endoscopy

Endoscopy was performed in the department of

gastroenterology in all selected cases to look for gastro-oesophageal varices and other associated signs of portal hypertension like red weal marks, cherry red spots. Grading of oesophageal varices was done according to Paqet[15]: Grade I – small varices without luminal prolapse; Grade II – moderate-sized varices showing luminal prolapse with minimal obscuring of the gastro-oesophageal junction; Grade III – large varices showing luminal prolapse substantially obscuring the gastro-oesophageal junction; Grade IV – very large varices completely obscuring the gastro-oesophageal junction.

Statistical analysis

Results were analysed by statistical methods like average, standard deviation, student's "t" test and Pearson's correlation co-efficient.

Results

A total of 82 patients with cirrhosis of liver were selected for the study. Among them, 56 were male (68%), the remaining were female (32%). Median age of the study group was 40 years; range 19 – 64 years.

In the study it has been found that 20 patients out of 82 had not developed gastro-oesophageal varices. Among the rest of the 62 patients, 19 had low-grade varices (grade I and II) and 43 patients had high-grade varices (grade III and IV).

Average serum albumin level of these 82 patients was 2.76 ± 0.585 gm/dl and globulin 3.898 ± 0.792 gm/dl. And average serum albumin level in variceal and non-variceal group was 2.52 ± 0.421 gm/dl and 3.484 ± 0.402 gm/dl respectively. Average platelet counts were 1,11,000 ± 2,840 and 2,15,000 ± 5,500/cu mm of blood in the same groups respectively.

Table I: Showing different values in the variceal and non-variceal group.

Parameters	Variceal group (n=62)	Non-variceal group (n=20)	P value
Average portal vein diameter (mm)	13.998±1.123	11.545±1.514	p<0.05
Average spleen size (cm)	14.997±1.922	13.129±1.102	p<0.001
Average platelet count (per/cumm)	1,11,000±2,840	2,15,000±5,500	p>0.10
Average albumin level (gm/dl)	2.52±0.421 gm/dl	3.484±0.402	p>0.10

Average portal vein diameter (PVD) of patients without gastro-oesophageal varices was 11.545 ± 1.514 mm and of patients with varices 13.998 ± 1.123 mm. This difference is statistically significant (t = 2.27517E-11; p < 0.05).

Average spleen size of patients without varices was 13.129 ± 1.102 cm and with varices 14.997 ± 1.922 cm. And this variation is also statistically significant (t = 9.12963E-05; p < 0.001)

Hence it had been found that gastro-oesophageal varices developed when PV diameter was > 11.5 mm and spleen size was > 13.1 cm.

It had also been found that there was a positive correlation between grading of oesophageal varices and portal vein

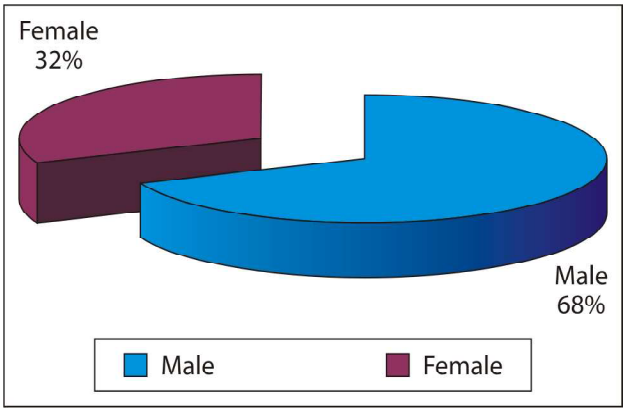

Fig. 1: Sex distribution.

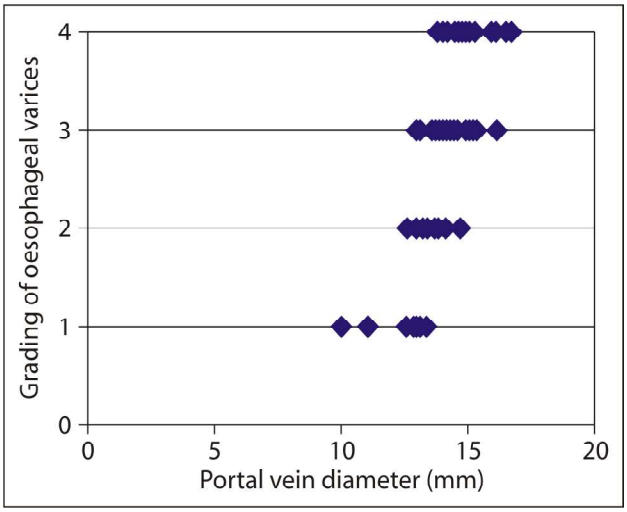

Fig. 2: Correlation of variceal grading with portal vein diameter.

diameter (r = 0.707) and it is statistically significant (p < 0.001). That means when portal vein diameter increases, oesophageal varix also increases in size.

There was also a positive correlation between splenic size and oesophageal grades (r = 0.467; p < 0.001). So, oesophageal varix also depends on spleen size.

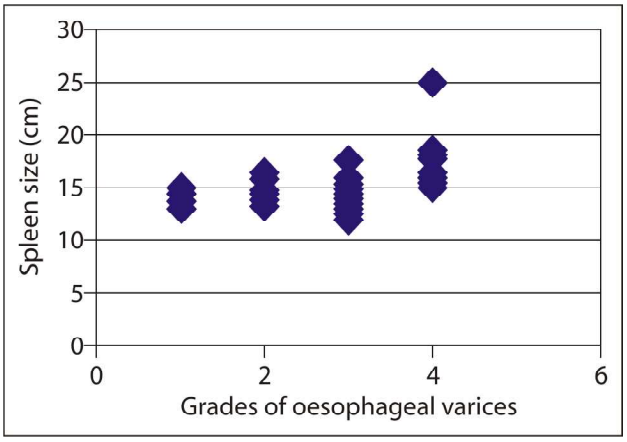

Fig. 3: *Correlation of spleen size with portal vein diameter.*

Discussion

A total of 82 patients were selected in our study; males – 56 and median age of the study population was 40 years, range being 19 to 64 years. In another Indian study by Sharma and Aggarwal[16], proportion of male patients (87 males out of 101 patients) was slightly higher than our study, but median age was more or less similar (median age 45 years) to ours.

Average serum albumin and platelet count was 2.52 gm/dl and 1,11,000/cu mm of blood respectively in the variceal group. Though the differnces of these values with those of the non-variceal group were not statistically significant, it had been found that these values corroborated to other studies. In the study of Thomopoulos *et al*[17] the patients with the varices had the platelet count less than 1,18,000/cu mm. Serum albumin level was less than 2.95 gm/dl in the variceal group as shown by the Sarwar *et al*[4].

Upper GI endoscopy of the study population revealed that a total of 62 patients had developed gastro-oesophageal varices and 20 patients were yet to develop these. Ultrasonography showed that average portal vein diameter (PVD) of the patients with gastro-oesophageal varices (GEV) was 13.998 ± 1.123 mm and without gastro-oesophageal varices (GEV-0) was 11.545 ± 1.514 mm. This difference was statistically significant (p < 0.05).

Radiologically, average spleen size of the patients with GEV was 14.997 ± 1.922 cm and spleen size in the GEV-0 group was 13.129 ± 1.102 cm, and the difference was highly significant (p < 0.001).

So, it can be concluded that gastro-oesophageal varices developed in cirrhotic patients with portal vein diameter more than 11.545 mm and larger than 13.1 cm spleen size.

These observations were more or less similar to other studies. In the study by Prihatini *et al*[18], portal vein diameter 11.5 mm and spleen size of 10.3 cm were predictive factors for oesophageal varices in liver cirrhosis. Here, spleen size was smaller than our study, but portal vein diameter was corroborative to ours. Portal vein diameter for development of gastro-oesophageal varices was also nearer to the Sarwar *et al*[4] study (portal vein 11 mm). Thomopoulos *et al*[17] showed that the majority of patients with gastro-oesophageal varices had spleen size more than 13.5 cm which was nearly similar to ours.

In our study, it was also found that in patients with gastro-oesophageal varices, grading of varices directly correlated with portal vein diameter and spleen size. (r = 0.707 and 0.467 respectively). That implied, when portal vein diameter and spleen size increased, gastro-oesophageal varices also transformed to higher grades. Average portal vein diameter and spleen size in higher grade varices were 14.43 ± 0.86 mm and 15.36 ± 2.14 cm. In a study by Schepis *et al*[19] portal vein diameter 13 mm was associated with higher grade varices. Sharma and Aggarwal[16] had noted that a clinically palpable spleen was associated with high-grade varices; however, they did not measure the splenic size radiologically.

Hence, it can be concluded that gastro-oesophageal varices in cirrhotic patients (without previous history of gastrointestinal bleeding), directly correlates with portal vein diameter and splenic size.

Conclusion

We can conclude that in cirrhosis of liver with portal

hypertension, without previous history of upper gastrointestinal bleeding:

- Portal vein diameter increases with development of varices.
- Spleen size increases with formation of gastro-oesophageal varices.
- There is a positive correlation between portal vein diameter ($r = 0.707$) and spleen size ($r = 0.467$) with gastro-oesophageal varices, which was the aim of our study.

Hence, measurement of portal vein diameter and splenic size by ultrasonography is a non-invasive predictive indicator of the development of gastro-oesophageal varices in cirrhosis of liver.

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ORIGINAL ARTICLE

JIACM 2011; 12(4) : 271-3

Keratomycosis – A retrospective study from a North Indian tertiary care institute

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Abstract

Introduction: *Fungal infections of the cornea continue to be an important cause of ocular morbidity, particularly in agricultural communities of the developing world.*

Method: *A retrospective study was carried-out to find the prevalence of mycotic keratitis in and around Amritsar, Punjab.*

Results: *72 (43.6%) corneal scrapings were positive on direct examination and 65 (39.3%) on culture. Most common fungal isolates were Aspergillus species (50%), Candida (20%), Fusarium (15%), Penicillium and Curvularia (9.7%), Paecilomyces and Mucor (5%). Exserohilum and Exophiala (1.3%) each.*

Conclusion: *Knowledge of geographical distribution of these fungi is important in deciding appropriate treatment.*

Introduction

Corneal ulceration is a major cause of monocular blindness in developing countries. Surveys in Asia and Africa have confirmed these findings[1, 2]. Ophthalmic mycosis is being increasingly recognised as an important cause of ocular morbidity and keratomycosis is the most frequent presentation. In tropical and subtropical countries, the incidence of mycotic keratitis is more than 50% of all culture-proven cases of keratitis[3]. Due to a large agrarian population and environmental factors, fungi contribute largely to the environmental list of infectious intruders of the cornea[4]. In most of the studies, corneal trauma is the commonest predisposing factor. Other predisposing factors could be occupation, rural background, prolonged use of topical corticosteroids or antimicrobial agents, systemic diseases such as diabetes mellitus, pre-existing ocular diseases, and use of contact lenses. The aetiological and epidemiological pattern of corneal ulceration varies significantly with the patient population, health of the cornea, geographical region, and also tends to vary with period of time[5]. Therefore, a retrospective study was done over a period of five years from January 2003 to December 2008 to find out the epidemiological features and the prevalence of keratomycosis in this area.

Material and method

A total of 165 patients with suspected fungal corneal ulcer presenting in the out-patient department of Ophthalmology were investigated for fungal aetiology in the department of Microbiology, Government Medical College, Amritsar, Punjab.

In all these patients, a detailed ocular examination was carried-out and corneal scrapings were collected by experienced ophthalmologists under all aseptic precautions. The samples were sent to the Microbiology department immediately. Direct microscopic examination of corneal scrapings was performed with 10% KOH wet mount and by Gram's staining for demonstration of fungal element. Special staining procedures such as calcofluor white staining or PAS staining could not be employed due to unavailability of these kits.

Another portion of corneal scrapings was inoculated on solid culture media such as Sabourauds agar (SDA) without cyclohexamide and blood agar. SDA was incubated at 25 °C ad 37 °C for four weeks and blood agar was incubated at 37 °C and examined daily and to be discarded after 7 days. These media were checked for any fungal growth in the form of yeast or mycilia daily. Growth was identified by standard procedures[6].

Results

Out of 165 cases of suspected fungal corneal ulcers, 72 (43.6%) were positive for fungal elements in KOH

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preparation; and these 72 when cultured, showed growth in 65 samples (39.3%)

The age-wise, sex-wise, occupation and predisposing factor-wise positivity is shown in Table I.

Table I:

Age (in years)	No. of cases	%
0 – 20	5	6.94
21 – 40	20	27.77
41 – ≥ 60 years	47	65.28
Sex		
Male	57	79.97
Females	15	20.83
Occupation: agriculture		
Field worker	60	83.33
Others	12	16.67
Predisposing factors		
Trauma	65	90.27
Over-use of antibiotics	45	62.50
Use of topical steroids	30	41.67
Diabetes	5	6.94
Tuberculosis	9	12.5

Fungal isolates are shown in Table II.

Table II:

Fungal isolates	No. of cases	%
Aspergillus	36	50.00
Candida	15	20
Fusarium	11	15
Penicillium	7	9.7
Curvularia	7	9.7
Paecilomyces	4	5
Mucor	4	5
Exserohilium	1	1.3
Exophiala	1	1.3

Discussion

Mycotic keratitis occurs much more frequently in developing countries such as India rather than developed countries[7]. Its incidence is reported to vary from 7 – 40% in various parts of the country[5]. In the present study, incidence was found to be 43.6% which is in accordance with other studies reported earlier from this part of the country[2, 4, 7]. On culture, seven samples remained sterile despite positive direct microscopic findings. These were considered positive because direct microscopic findings corroborated with the clinical findings of the patients. The reason for cultures to be sterile even when direct microscopy was positive could be that the patients were already using topical steroids or antifungal agents before the corneal scraping samples were taken. Apart from this, many times the samples were so insufficient in quantity that only KOH mount preparation could be feasible and inadequate material was left for establishing cultures.

In the present study, the incidence was highest in the over 40 years age group (Table I). It has been reported to be 77% by other workers[8]. In addition, keratomycosis was found to be more common in men than women. Male to female ratio observed was 3:1 in this study. Similar findings have been reported earlier[6, 9]. The reason for this is that men in this age group have greater exposure to the fungal agents due to maximum outdoor activity.

Corneal injury was an important predisposing factor. We have obtained a definite history of antecedent corneal injury in about 90% of patients which is in agreement with the findings of other workers who reported it to be 80 – 90%[2,8,10]. Other predisposing risk factors were chronic antibiotic usage (62.5%) and use of topical corticosteroids (41.6 %). The reason for this could be easy over-the-counter availability of these antibiotics and steroids eye drops in our country. Moreover, due to illiteracy, patients keep on using these eye drops continuously for long periods, many a times even without a prescription. In the present study, 14 patients gave history of systemic diseases such as diabetes mellitus and tuberculosis. In ten cases, no predisposing factor could be identified. In the present study, not even a single person reported the usage of contact lenses. This is in contrast to a study from Philadelphia[11] in which the three most common risk factors were found to be chronic ocular surface disease, contact lens usage, and use of topical corticosteroids; and interestingly, *Candida albicans* was the most common isolate (46%).

In the present study, *Aspergillus* species have been

reported, which is in consonance with recent studies from the Indian subcontinent[12,13] (Table I).

Aspergillus fumigatus was the commonest infective agent. *Fusarium* and *Candida albicans* have been reported as predominant agents[9,11,14,15]. *Penicillium* and *Curvularia* were isolated in 9.7% whereas other workers reported these to be 8.82%[7]. Other studies showed isolation of *Alternaria*, *Paecilomyces*, *Mucor* from corneal ulcers which is similar to results of the present study[16,17,18] (Table II). *Exophiala* and *Exserohilum* were reported to be 2.5% in present study. These were earlier reported by Patel *et al* and Peerpur *et al*[18,19]. To the best of our knowledge, not many studies are available on these; or its isolation rate is very low.

Conclusion

Mycotic keratitis continues to be an important cause of ocular morbidity, mainly in persons inhabiting rural areas, involved in outdoor and agricultural activity. Young male adults affected in these circumstances are often the bread winners of their family, and blindness in them leads to grave economic consequences. Early, meticulous examination of corneal scrapings by direct microscopy, and timely institution of antifungal therapy may limit ocular morbidity and its disastrous sequelae among these patients.

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ORIGINAL ARTICLE

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Study of antimicrobial sensitivity pattern of Gram-positive CSF isolates among children suffering from septic meningitis in a tertiary care hospital

Y Chugh*, AK Kapoor**, N Kastury***, AK Srivastava****, A Bhargava*****, A Sharma*

Abstract

Aims of the study: The present study was undertaken to assess the antimicrobial susceptibility patterns of Gram-positive CSF isolates of septic meningitis at a tertiary care hospital. All over the world, indiscriminate and irrational use of antimicrobials has led to the development of antimicrobial resistance owing to significant changes in microbial genetic ecology.

Methodology: 3 – 5 ml of CSF sample was collected by lumbar puncture from all admitted children (up to 12 years) clinically suspected to be suffering from septic meningitis. Identification of bacterial isolates was done by Gram's staining, motility, colony characteristics, and biochemical tests. Microbial sensitivity testing was done by using the Kirby-Bauer's disk diffusion method following NCCLS guidelines.

Results: Of 638 CSF samples collected from clinically suspected cases of septic meningitis, only 102 samples (15.99%) were culture positive. 505 samples (79.15%) were sterile. 63 (61.76%) were males and 39 (38.24%) females. M: F ratio was 1.62: 1. Maximum culture-positive samples, i.e., 45 (44.12%) were from children (1 – 12 years) and the least, i.e., 21 (20.59%) samples belonged to the infant age group (1 month – 1 year). Maximum incidence was recorded during summer-rainy season (51 cases) and in institutional delivery cases. Primary immunisation of children did not protect against septic meningitis. 66 (64.71%) cases were of Gram-positive and 36 (35.29%) were Gram-negative. Of 66 Gram-positive isolates, 36 (54.55%) were of Streptococcus spp., 24 (36.36%) Staphyloccus aureus, and 6 (9.09%) cases were of Coagulase-negative Staphylococcus (CONS). Gram-positive isolates were highly sensitive (100%) to linezolid and vancomycin followed by piperacillin-tazobactam (95.45%). Maximum resistance was observed with cotrimoxazole (86.36%) followed by tetracycline (84.85%).

Conclusion: It is concluded that Gram-positive isolates are highly sensitive (100%) to linezolid and vancomycin followed by piperacillin-tazobactam combination. Staphylococcus was also highly sensitive to pristinamycin as well. Initially, piperacillin-tazobactam should be used as broad spectrum empirical therapy while linezolid and vancomycin should be kept as reserve drugs for severe nosocomial infections. Periodic susceptibility testing should be carried out over a period of 2 – 3 years to detect resistance trends. Besides, rational prescription of antimicrobial agents and formulation of an empirical therapy based on trends of resistance is to be evolved.

Key words: Antimicrobial susceptibility, combination antimicrobials, Gram-positive microorganisms.

Introduction

Bacterial meningitis is one of the most potentially serious infections occurring in infants and older children and is associated with a high incidence of acute complications and risk of long-term morbidity[1]. Systemic bacterial infections are known by the generic term "neonatal sepsis" which incorporates septicaemia, pneumonia, and meningitis of the new born. Neonatal sepsis is the single most important cause of neonatal deaths in the community, accounting for over half of them. About one-third of the neonates with septicaemia may have coexistent meningitis[2].

Despite great advances in antimicrobial therapy, neonatal and paediatric life support measures and early detection of risk factors, bacterial sepsis, and meningitis continue to be a major cause of morbidity and mortality in newborns, particularly in low-birth-weight infants[3]. A wide variety of spectrum of organisms has been described for cases of septic meningitis and this spectrum is subjected to geographical alterations. The organisms isolated are more often resistant to multiple antimicrobials, making treatment more difficult and leading to grave sequelae. This calls for the need of bacteriological monitoring in paediatric wards and their antibiotic sensitivity pattern. Neonates are particularly vulnerable to infections, so any delay in the initiation of empirical therapy or wrong choice of antibiotic could be

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fatal. Antibiotics are usually administered before the laboratory results of CSF culture and sensitivity are available. To ensure appropriate therapy, current knowledge of the organisms that cause septic meningitis and their antibiotic susceptibility pattern in a particular setting or region is of utmost importance.

The initial antibiotic regimen should be such that it covers all the likely pathogens according to the age of the child; it should achieve sufficient bactericidal concentration in the CSF and the combination of antimicrobials should not be antagonistic. Later the treatment can be modified depending upon the results of Gram's stain and CSF culture. CSF culture provides a confirmatory evidence of acute bacterial meningitis (ABM) and is essential for selecting appropriate antibiotic(s) for the aetiological organisms[4]. The present study has been undertaken to evaluate the spectrum of pathogens causing septic meningitis and their antimicrobial susceptibility pattern in indoor patients of clinically suspected ABM, also known as septic meningitis (SM).

Material and methods

Our study group comprised of all children aged upto 12 years, of either sex who were clinically suspected to be suffering with septic meningitis and admitted to S.N. Children Hospital attached to M.L.N. Medical College, Allahabad during the study period (2007 – 08). Patients diagnosed repeatedly with events of bacterial meningitis due to structural defects of the central nervous system and cases of meningitis caused by *Mycobacterium tuberculosis* were excluded[5].

3 – 5 ml of CSF was collected by lumbar puncture under complete aseptic precautions (by a well-trained physician) in a sterile screw-capped container. Rate of collection was 3 – 5 drops/minute[6]. The specimen of CSF was transported to the microbiology laboratory as soon as possible – usually within 30 minutes – with all proposed precautions, since delay may result in the death of delicate pathogens such as *meningococci*, and the disintegration of leucocytes. Sample was not kept in a refrigerator which tends to kill *H. influenzae*. If delay for a few hours was unavoidable, the specimen was kept in an incubator at 37 °C[6]. It may be emphasized that CSF should be collected, prior to administration of antimicrobials, from all cases of suspected meningitis and should be sent for culture and sensitivity examination timely for better results.

A portion of the sample was inoculated on blood agar, chocolate agar, and McConkey agar by standard loop method. The culture plate was incubated under 5 – 10% CO_2 at 37 °C for 18 – 24 hours. The remaining CSF sample was inoculated in 5 ml brain-heart infusion broth and then incubated overnight. Next day it was observed for turbidity. If turbidity appeared and there was no growth on any of the solid media then a subculture was done from brain heart infusion broth on the three solid media mentioned as above.

The bacterial growth was interpreted by semi-quantitative method in terms of heavy, moderate and scanty growth on primary plating. No quantification was done if growth was observed after enrichment. Any specimen that showed no bacterial growth after 48 hrs of incubation in any of the four culture media was labelled as 'sterile'. Cultures showing growth of *Candida* spp. were not evaluated further.

Identification of bacterial isolates was done by Gram's staining, motility, colony characteristics and biochemical tests. Antibiotic susceptibility tests and interpretations were carried-out for bacterial isolates by the Kirby-Bauer's disc diffusion method following NCCLS guidelines[7,8,9].

The test was done by applying commercially available (HiMedia Laboratories Pvt. Limited, Mumbai, India) filter paper discs impregnated with a specific amount of an antibiotic on to Mueller-Hinton Agar/Blood agar surface, over which a saline suspension of microorganism had been poured.

The strains under test were reported as *sensitive*, *moderately sensitive* or *resistant* comparing the diameter of zone of inhibition to the standard antimicrobial sensitivity chart.

Observations and results

Clinical presentation(s) of the patient of septic meningitis at the time of admission showed marked variability and

there might be more than one clinical signs and symptoms. Among neonates, abnormal body temperature – either fever or hypothermia – was the most frequent presentation in 25 cases, followed by convulsions in 22, and refusal to feed in 19. Among infants, convulsions were the most frequent symptom in 19, followed by fever 18, shrill cry 17, irritability 15, vomiting 10, and anterior fontanel bulging in 8 cases. While in children, the most frequent symptom was convulsions in 42, followed by fever (40), altered sensorium (39), headache (27), vomiting (24), neck rigidity (18) and photophobia in 11 cases.

CSF samples from 638 clinically suspected cases of septic meningitis were collected. Of these, 102 (15.99%) samples displaying single bacterial growth – termed as 'Culture Positive' – were included in the study. No sample was found to have two bacterial growths simultaneously. 505 (79.15%) samples showed no bacterial growth (sterile), and 31 (4.86%) exhibited three or more bacterial growths (grossly contaminated), and altogether these 536 (84.01%) samples were discarded for the purpose of the study. It was noted that the rate of bacterial isolation was affected by antibiotic use prior to lumbar puncture, and that rate was increased if direct plating of CSF was done at bedside.

Out of 102 culture positive samples, 63 (61.76%) were males and 39 (38.24%) females with the M: F ratio 1.62: 1 exhibiting predominance of males. Maximum number of culture-positive CSF samples 45 (44.12%) were from children of age group 1 – 12 years with M: F ratio of 0.67: 1; this was followed by neonatal age group (0 – 28 days) with 36 (35.29%) culture positive samples and M: F ratio of 11: 1. Least number of samples, i.e., 21 (20.59%) were found culture-positive from the infant age group (1 month-1 year) with M: F ratio of 1.33: 1.

Maximum incidence of 51 cases was noted during 4 months of May to August (summer-rainy season) as compared to 15 cases during 4 months of winter season (September to December) suggesting more prevalence of septic meningitis during summer-rainy season. Place and mode of delivery as well as vaccination status has been shown in Table I. 58 institutionally delivered cases compared to 44 home delivered cases were found culture-positive. Incidence was more in vaginally delivered cases compared to caesarean cases. More involvement of vaccinated cases was noted.

Of the 102 culture-positive cases, 66 (64.71%) were Gram-positive and 36 (35.29%) cases Gram-negative, suggesting a predominance of Gram-positive cases in our study. Of the 66 Gram-positive isolates, maximum 36 (54.55%) were of *Streptococcus spp.* followed by *Staphylococcus aureus* in 24 (36.36%) and Coagulase-negative *Staphylococcus* (CONS) in 6 (9.09%) cases.

Sensitivity pattern of *Streptococcus spp.*, *Staphylococcus aureus and* Coagulase-negative *Staphylococcus* (CONS) are given in respectively Table II, III, and IV.

Overall Gram-positive isolates were highly sensitive (100%) to linezolid and vancomycin followed by piperacillin-tazobactam (95.45%), amikacin (90.91%), cefoperazone-sulbactam (86.36%), meropenem (86.36%) and pristinamycin (83.33%). Maximum resistance was observed with cotrimoxazole (86.36%), followed by tetracycline (84.85%) and penicillin G (69.7%).

Discussion

Septic meningitis is one of the most important causes of morbidity and mortality among children including neonates. A wide spectrum of microorganisms has been described for the cases of septic meningitis and this spectrum is subjected to geographical alterations. Two basic problems in reference to septic meningitis are firstly non-specific clinical features and difficult laboratory confirmation making the diagnosis difficult, and secondly neonates are particularly vulnerable to infections as they are beset with an immature immune system. Moreover, the organisms isolated are more often resistant to multiple antimicrobials, which make the treatment more difficult, leading to consequences such as increased hospital stay as well as mortality. Multiple antimicrobial resistance in bacterial population is a pervasive and growing clinical problem, thus a major threat to life in septic meningitis cases. Therefore, area specific bacteriological monitoring studies aimed to gain knowledge about the type of pathogen responsible for septic meningitis and their susceptibility patterns may help clinicians to choose correct empirical treatment. This study will not only throw light about the spectrum of microorganisms isolated from CSF in cases with septic meningitis but also in evaluating antimicrobial sensitivity pattern to formulate empirical antimicrobial therapy.

Table I: Place, mode of delivery, and vaccination status of septic meningitis cases (N = 102).

Gram's stain	Place of delivery		Mode of delivery		Vaccination status	
	Institutional No. (%)	Home No. (%)	Vaginal* No. (%)	Caesareans No. (%)	Immunised No. (%)	Non-immunised No. (%)
Gm+ve N_1 = 66 (64.71)	39 (38.24)	27 (26.47)	50 (49.02)	16 (15.69)	49 (48.04)	17(16.67)
Gm-ve N_2=36 (35.29)	19(18.63)	17 (16.67)	29 (28.43)	7 (6.86)	25 (24.51)	11(10.78)
Total N=102 (100)	58(56.86)	44 (43.14)	79 (77.45)	23 (22.55)	74 (72.55)	28 (27.45)

**Place of delivery in vaginal mode may be home or institutional.*

Table II: Sensitivity pattern of *Streptococcus spp.* to various antimicrobial agents (N = 36)

S. No.	Antibiotic	No. of cases (% Sensitivity)		
		S	MS	R
1	Amikacin	33 (91.67)	0 (0)	3 (8.33)
2	Gentamicin	21 (58.33)	3 (8.33)	12 (33.33)
3	Cefepime	33 (91.67)	0 (0)	3 (8.33)
4	Cefotaxime	19 (52.78)	4 (11.11)	13 (36.11)
5	Cefuroxime	18 (50)	1 (2.78)	17 (47.22)
6	Ceftazidime	20 (55.56)	4 (11.11)	12 (33.33)
7	Ceftriaxone	25 (69.44)	4 (11.11)	7 (19.44)
8	Meropenem	30 (83.33)	0 (0)	6 (16.67)
9	Oxacillin	23 (63.89)	0 (0)	13 (36.11)
10	Penicillin G	13 (36.11)	0 (0)	23 (63.89)
11	Amoxicillin-Clavulanic Acid	28 (77.78)	0 (0)	8 (22.22)
12	Cefoperazone-Sulbactam	30 (83.33)	0 (0)	6 (16.67)
13	Piperacillin-Tazobactam	36 (100)	0 (0)	0 (0)
14	Gatifloxacin	27 (75)	3 (8.33)	6 (16.67)
15	Levofloxacin	23 (63.89)	3 (8.33)	10 (27.78)
16	Chloramphenicol	10 (27.78)	0 (0)	26 (72.22)
17	Co-trimoxazole	4 (11.11)	0 (0)	32 (88.89)
18	Linezolid	36 (100)	0 (0)	0 (0)
19	Pristinamycin	25 (69.44)	0 ()	11 (30.56)
20	Tetracycline	3 (8.33)	0 (0)	33 (91.67)
21	*Vancomycin*	*36 (100)*	*0 (0)*	*0 (0)*

**S = Sensitive; MS = Moderately sensitive; R = Resistant.*

The study observed an age-wise variability in symptomatology at the time of admission amongst neonates, infants, and children. Thus, among neonates, abnormal body temperature – either fever or hypothermia – was most frequent presentation, followed by convulsions and refusal to feed. Other workers in the field also noted similar spectrum of presentation. Among infants, convulsion was the most frequent symptom, followed by fever, shrill cry, irritability, vomiting, and anterior fontanelle bulging. While in children, the most frequent symptom was convulsion, followed by fever, altered sensorium, headache, vomiting, neck rigidity and

Table III: Sensitivity pattern of *Staphylococcus aureus* to various antimicrobial agents (N = 24).

S. No.	Antibiotic	No. of cases (% Sensitivity)		
		S	MS	R
1	Amikacin	21 (87.5)	0 (0)	3 (12.5)
2	Gentamicin	17 (70.83)	0 (0)	7 (29.17)
3	Cefepime	15 (62.5)	0 (0)	9 (37.5)
4	Cefotaxime	12 (50)	0 (0)	12 (50)
5	Cefuroxime	16 (66.67)	0 (0)	8 (33.33)
6	Ceftazidime	18 (75)	0 (0)	6 (25)
7	Ceftriaxone	15 (62.5)	0 (0)	9 (37.5)
8	Meropenem	21 (87.5)	0 (0)	3 (12.5)
9	Oxacillin	8 (33.33)	0 (0)	16 (66.67)
10	Penicillin G	5 (20.83)	0 (0)	19 (79.17)
11	Amoxicillin-Clavulanic Acid	18 (75)	0 (0)	6 (25)
12	Cefoperazone-Sulbactam	21 (87.5)	0 (0)	3 (12.5)
13	Piperacillin-Tazobactam	21 (87.5)	0 (0)	3 (12.5)
14	Gatifloxacin	18 (75)	0 (0)	6 (25)
15	Levofloxacin	12 (50)	6 (25)	6 (25)
16	Chloramphenicol	7 (29.17)	3 (12.5)	14 (58.33)
17	Co-trimoxazole	4 (16.67)	0 (0)	20 (83.33)
18	Linezolid	24 (100)	0 (0)	0 (0)
19	Pristinamycin	24 (100)	0 (0)	0 (0)
20	Tetracycline	4 (16.67)	0 (0)	20 (83.33)
21	Vancomycin	24 (100)	0 (0)	0 (0)

**S = Sensitive; MS = Moderately sensitive; R = Resistant.*

photophobia. It may be emphasised that one patient might have more than one clinical signs and symptoms at the time of admission. Thus, 102 cases depicted a total of 390 signs and symptoms at the time of admission. Our findings corroborate with those of Salam[10] who observed that children of all ages (newborn to 12 years) attended the paediatric emergency with fever, convulsions and altered sensorium.

Importantly, institutionally delivered cases are more prone to septic meningitis compared to domestic deliveries despite primitive facilities and unhygeinic conditions prevailing in latter situations. This is probably due to increased prevalence of nosocomial infections in hospital settings. Further, incidence was more in vaginally delivered cases compared to caesarean cases.

More sterile cultures in the present study may be due to the fact that some organisms cannot survive more than an hour's delay in transportation; hence, there should be better training for proper sample handling and its timely transportation. 31 samples were found to be grossly contaminated indicating the relevance of proper aseptic precautions by the clinician.

The culture positivity in our study was 15.99%. Our findings are in conformity with those of Kalghatgi *et al*[11] (15%), Sonavane *et al*[12] (ranges between 6 - 50%), and at variance with those of Surinder *et al*[13] (23.1%), Salam[10] (27.27%), Singhi *et al*[14] (30%). In contrast, a higher culture-positivity was reported by Al Khosarani and Banajeh[15] (95.6%), Mani *et al*[16] (73.8%) and Theodoridou *et al*[5] (53.7%). This is primarily because of previous antibiotic

intake before the patient reports to a tertiary care hospital. Also, there are many other clinical conditions like aseptic meningitis, tuberculous meningitis, etc., which clinically simulate septic meningitis and need to be differentiated.

infants age group (62.5%) which was in contrast to our findings of least involvement of infants (20.59%). Sigauque *et al*[18] reported that incidences were more than three times higher in < 1 year age group which was at much

Table IV: Sensitivity pattern of coagulase negative *Staphylococcus* (CONS) to various antimicrobial agents (N = 6).

S. No.	Antibiotic	No. of cases (% Sensitivity)		
		S	MS	R
1	Amikacin	6 (100)	0 (0)	0 (0)
2	Gentamicin	5 (83.33)	0 (0)	1 (16.67)
3	Cefepime	3 (50)	0 (0)	3 (50)
4	Cefotaxime	5 (83.33)	1 (16.67)	0 (0)
5	Cefuroxime	4 (66.67)	2 (33.33)	0 (0)
6	Ceftazidime	6 (100)	0 (0)	0 (0)
7	Ceftriaxone	6 (100)	0 (0)	0 (0)
8	Meropenem	6 (100)	0 (0)	0 (0)
9	Oxacillin	6 (100)	0 (0)	0 (0)
10	Penicillin G	2 (33.33)	0 (0)	4 (66.67)
11	Amoxicillin-Clavulanic Acid	6 (100)	0 (0)	0 (0)
12	Cefoperazone-Sulbactam	6 (100)	0 (0)	0 (0)
13	Piperacillin-Tazobactam	6 (100)	0 (0)	0 (0)
14	Gatifloxacin	6 (100)	0 (0)	0 (0)
15	Levofloxacin	6 (100)	0 (0)	0 (0)
16	Chloramphenicol	5 (83.33)	0 (0)	1 (16.67)
17	Co-trimoxazole	0 (0)	1 (16.67)	5 (83.33)
18	Linezolid	6 (100)	0 (0)	0 (0)
19	Pristinamycin	6 (100)	0 (0)	0 (0)
20	Tetracycline	3 (50)	0 (0)	3 (50)
21	Vancomycin	6 (100)	0 (0)	0 (0)

**S = Sensitive; MS = Moderately sensitive; R = Resistant.*

Our finding in respect to predominance of male involvement (M: F ratio 1.62:1) was in agreement with those of Keshari *et al*[17] (M: F ratio 1.7:1) and Sonavane *et al*[11] (1.35:1) and was at variance to those of Singhi *et al*[14] (M: F ratio of 3.21: 1). It is of relevance that in the present study, the M: F ratio in different age groups was found to be quite variable. Thus, culture-positive cases in neonatal age groups were predominantly males with M: F ratio of 11:1.

Singhi *et al*[14] reported that maximum cases were from

variance to our findings.

Maximum incidence (51 cases) was seen during the summer and rainy season, i.e., from May to August. Our findings were in contrast to those by Farag *et al*[19] who reported maximum incidence in winter season (fall 35.1% and winter 48.5%) in Egypt. They observed that this was in families with high crowding index. This probably may be due to geographial variation. While in tropical countries like India, the incidence of infection is usually more during summer and rainy seasons.

In the present study, we noted a predominance (64.71%) of Gram-positive organisms. Sigauque *et al*[18] (57.75%), Mani *et al*[16] (65.9%) also reported a predominance of Gram-positive organisms while other workers[11,13,15,17,21] reported a predominance of Gram-negative organisms, the incidence ranging from 58.69% to 80.93%.

A 100% correlation between Gram's staining and culture was recorded. Our findings were in conformity with those of Sonavane *et al*[12] who also reported a 100% correlation between Gram's staining and culture. Dunbar *et al*[20] reported that CSF Gram's stain was 92% sensitive and concluded that microscopic examination of Gram's stained, concentrated CSF is highly sensitive and specific in early diagnosis of bacterial or fungal meningitis. In contrast, Surinder *et al*[13] reported that Gram's stain and culture showed 16.9% and 23.1% positivity respectively, i.e., correlation of 73.16%. Thus Gram's stain was a gold standard method to access the causative agent of septic meningitis.

Of 66 Gram-positive isolates, maximum were *Streptococcus spp.* 36 (54.55%), followed by *Staphylococcus aureus* (36.36%) and Coagulase negative *Staphylococcus* (CONS) (9.09%) cases. Tang *et al*[21] also reported that *Streptococcus spp.* (71.1%) were the most common causative organism supporting our observations. In variance Mani *et al*[16] reported *Streptococcus spp.* (97.27%), and *Staphylococcus* (2.73%); and Sigauque *et al*[18] observed *Streptococcus* spp. (92.67%), and *Staphylococcus* (7.33%).

In our study, overall, the Gram-positive isolates were highly sensitive (100%) to linezolid and vancomycin followed by piperacillin-tazobactam (95.45%), amikacin (90.91%), cefoperazone-sulbactam (86.36%), meropenem (86.36%) and pristinamycin (83.33%). Maximum resistance was observed to co-trimoxazole (86.36%), followed by tetracycline (84.85%) and penicillin G (69.7%). In conformity to our findings, Gupta and Jain[22] reported that Gram-positive isolates were sensitive to vancomycin and ceftriaxone. The authors noted that Gram-positive isolates were also sensitive to penicillin G contrary to our findings. Al Khosarani and Banajeh[15] supported our observations that Gram-positive isolates were resistant to penicillin G. Kapil[23] reported that the isolates showed total resistance to penicillin and were also multi-drug-resistant namely to cefotaxime, erythromycin, chloramphenicol and Trimethoprim-sulphamethoxazole. Further, Shah and Narang[24] reported that Gram-positive organisms were highly susceptible to meropenem including Staphylococci (penicillinase negative and positive), coagulase-negative staphylococci (CONS), Streptococci, *Enterococcus.* The order of susceptibility was meropenem (99% susceptible) > piperacillin/tazobactam (77%) > ciprofloxacin (43%) > aminoglycosides and other β-lactams (30 – 40%).

Considerable variability in respect to culture and sensitivity of *Streptococcus* was reported. We observed 100% sensitivity with linezolid, vancomycin and piperacillin-tazobactam. Enting *et al*[25] reported that all streptococcal isolates were susceptible to ceftriaxone, a third generation cephalosporin. Gupta and Jain[22] also reported that *Streptococcus spp.* were sensitive to vancomycin, ceftriaxone and ciprofloxacin. Sonavane *et al*[12] observed that *Streptococcus spp.* was sensitive to amikacin and vancomycin. Our findings contradicted to those of Sigauque *et al*[18] who reported 93% streptococcal susceptibility to chloramphenicol probably due to geographical variations in the susceptibility of organisms. However, contrasting views were expressed by Kulkarni *et al*[26] that all the streptococcal isolates were sensitive to ampicillin, erythromycin, penicillin followed by chloramphenicol and were resistant to gentamicin, followed by tetracycline 94.4% and kanamycin 88.8%.

Staphylococcal isolates were 100% sensitive to linezolid, pristinamycin, and vancomycin. These isolates were also highly sensitive to amikacin, meropenem, piperacillin-tazobactam and cefoperazone-sulbactam (87.5% each). High resistance to tetracycline (83.33%), co-trimoxazole (83.33%) and penicillin G (79.17%) was observed. (Table III) Our findings were in agreement with other workers[24,22,15] who reported that Staphylococci (both penicillinase negative and positive) were highly susceptible to meropenem.

In our study, all strains of coagulase-negative *Staphylococcus* (CONS) were 100% sensitive to amikacin, ceftazidime, ceftriaxone, meropenem, oxacillin, amoxicillin-clavulanic acid, cefoperazone-sulbactam, piperacillin-tazobactam, gatifloxacin, levofloxacin, linezolid, pristinamycin and vancomycin. This was followed by gentamicin, cefotaxime and chloramphenicol (83.33% each). These isolates were resistant to co-trimoxazole

(83.33%), penicillin G (66.67%), cefepime (50%) and tetracycline (50%) (Table IV). In support to our study, Shah and Narang[24] stated that CONS were susceptible to meropenem, but they noted that methicillin-resistant strains of CONS and Staphylococci were not susceptible to meropenem and β-lactam antibiotics. Al Khosarani and Banajeh[15] also supported our findings about susceptibility of these pathogens to various cephalosporins.

It may be stated that moderately sensitive microbes become sensitive with high doses of antimicrobials. So, if we have no option left of using highly sensitive antimicrobials, due to resistance or hypersensitivity to particular antimicrobial agent, we can judiciously increase the dose of the moderately sensitive drug which may be effective.

As septic meningitis is an emergency medical condition, an empirical therapy must be instituted immediately after CSF collection which should be broad spectrum and sensitive against most of the prevailing causative microbes, without waiting for culture and sensitivity results. According to observations of our study an 'Empirical Therapy' should be a combination of IV 'piperacillin-tazobactam' or 'cefoperazone-sulbactam' as these agents were found to be effective against most of the causative pathogens (> 85%) whether Gram-positive or Gram-negative. Further, some drugs should be kept as 'reserve drugs', such as; 'linezolid' and 'vancomycin' for Gram-positive pathogens, and 'meropenem' for both Gram-positive as well as Gram-negative microbes. Once results of culture and susceptibility will be available, a 'definitive therapy' can be imparted depending upon specific causative pathogen cultured and its sensitivity. This will be cost effective also. Oral antimicrobials can be started when patient becomes well alert and able to take oral medicines. Maximum isolates were *Streptococcus pneumoniae* so it may be advised that for prevention of meningitis among children 'pneumococcal vaccine' should be included with primary immunisation in the National Immunisation Programme, and if not possible, it must be given at least to children at high-risk. Since it was observed that primary immunisation has no protective role towards septic meningitis.

High resistance to antimicrobial agents was due to irrational and irrelevant use of antimicrobial agents. The emergence of multi-drug resistant strains of Gram-negative bacteria and Gram-positive organisms is more worrisome in the present therapeutic scenario. Resistance to some agents can be overcome by modifying the dosage regimens or inhibiting the resistance mechanism (e.g., beta-lactamase inhibitors), whereas other mechanisms of resistance can only be overcome by using an agent from a different class. It is highly recommended that practicing physicians should become aware of the magnitude of existing problem of antibacterial resistance and help in fighting this deadly threat by rational prescribing.

The study has helped in evolving a panel of antimicrobial agents to be used in testing the susceptibility of individual Gram-positive organism.

In conclusion, it may be emphasised that there is utmost need to conduct area specific monitoring studies to profile different pathogens responsible for septic meningitis and their resistance patterns so as to generate data that would help clinicians to choose the correct empirical treatment.

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ROZAVEL F

REVIEW ARTICLE

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The charisma of "Magic Bullets"– Monoclonal antibodies (mAB/moAB) in clinical medicine

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Abstract

Monoclonal antibodies (mAb or moAb) are monospecific antibodies that are identical because they are produced by one type of immune cell that are all clones of a single parent cell. These antibodies have the unique property of specificity. It is possible to create monoclonal antibodies that specifically bind to a substance and they can then serve to detect or purify that substance. This has become an important tool in biochemistry, molecular biology, and medicine. When used as medications, the generic name ends in –mAB (the nomenclature of monoclonal antibodies is a naming scheme for assigning generic, or nonproprietary, names to a group of medicines called monoclonal antibodies. This scheme is used for both the World Health Organization's International, Non-proprietary Names and the United States Adopted Names[1,2]. In general, suffixes are used to identify a class of medicines; all monoclonal antibody pharmaceuticals end with the suffix –mAB. However, different infixes are used depending on the structure and function of the medicine).

Key words: Monoclonal, antibody, nomenclature, immune cell, clone.

Introduction

What are monoclonal antibodies?

Monoclonal antibodies are antibodies which have been artificially produced against a specific antigen. They are extremely specific and bind to their target antigens. In the laboratory, monoclonal antibodies are produced from the clones of one cell. That is why they are called "monoclonal". This basically means that every monoclonal antibody produced by this cell is exactly the same. This gives them the collective specificity that can be used in diagnosis and treatment. It is because of this extreme specificity they are also called "magic bullets". These cells have two important properties, one being capacity to produce antibodies and the other being immortality.

A brief history

Paul Ehrlich was the hero who first proposed the idea of a "magic bullet" at the beginning of the 20th century. He postulated that if a compound could be made that selectively targets a disease-causing organism, then a toxin for that organism could be delivered along with the agent of selectivity.

In the 1970s, the B-cell cancer multiple myeloma was known, and it was understood that these cancerous B-cells all produce a single type of antibody (a paraprotein). This was used to study the structure of antibodies, but it was not yet possible to produce identical antibodies specific to a given antigen.

In 1973, Jerrold Schwaber described a process of producing monoclonal antibodies involving human-mouse hybrid cells[3], and this process remains widely cited among those using human-derived hybridomas[4]. A science history paper on the subject gave some credit to Schwaber for inventing a technique that was widely cited, but stopped short of suggesting that he had been cheated[5]. The invention is generally credited to Georges Köhler, Céser Milstein, and Niels Kaj Jerne in 1975[6] who shared the Nobel Prize for Physiology or Medicine in 1984 for the discovery. The key idea was to use a line of myeloma cells that had lost their ability to secrete antibodies, come up with a technique to fuse these cells with healthy antibody producing B-cells, and be able to select for the successfully fused cells.

In 1988, Greg Winter and his team pioneered the techniques to humanise monoclonal antibodies[7].

Antibodies have two very useful characteristics. First, they are extremely specific; that is, each antibody binds to and attacks one particular antigen. Second, some antibodies, once activated by the occurrence of a disease, continue to confer resistance against that disease. It is the first trait

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of antibodies, their specificity, that makes monoclonal antibody technology so valuable. Not only can antibodies be used therapeutically to protect against disease, they can also help to diagnose a wide variety of illnesses, and can detect the presence of drugs, viral and bacterial products, and other unusual or abnormal substances in the blood.

Given such a diversity of uses for these disease-fighting substances, their production in pure quantities has long been the focus of scientific investigation. The conventional method was to inject a laboratory animal with an antigen and then, after antibodies had been formed, they were collected from the blood or serum. There are two problems with this method: It yields antiserum that contains undesired substances, and it provides a very small amount of usable antibody.

The basic principle

Monoclonal antibody technology produces a large amount of pure antibodies in the following ways:

1. We can obtain cells that produce antibodies naturally
2. We also have available a class of cells that can grow continually in cell culture;
3. If we form a hybrid that combines the characteristic of "immortality" with the ability to produce the desired substance, we would have, in effect, a factory to produce infinite antibodies of a single clone.

In monoclonal antibody technology, tumour cells that can replicate endlessly are fused with mammalian cells that produce an antibody. The result of this cell fusion is a "hybridoma", which will continually produce antibodies. These antibodies are called monoclonal because they come from only one type of cell, the hybridoma cell. A myeloma is a tumour of the bone marrow that can be adapted to grow permanently in cell culture. When myeloma cells were fused with antibody-producing mammalian spleen cells, it was found that the resulting hybrid cells, or hybridomas, produced large amounts of monoclonal antibodies. This product of cell fusion combined the desired qualities of the two different types of cells: the ability to grow continually, and the ability to produce large amounts of pure antibodies. Because selected hybrid cells produce only one specific antibody, they are more pure than the polyclonal antibodies produced by conventional techniques. They are potentially more effective than conventional drugs in fighting disease, since drugs attack not only the foreign substance but the body's own cells as well, sometimes producing undesirable side-effects such as nausea and allergic reactions. Monoclonal antibodies attack only the target molecule, with no or greatly diminished side-effects. Not only does this provide the basis for protection against disease organisms, but it makes antibodies attractive candidates to target other types of molecules found in the body, such as:

1. Receptors or other proteins present on the surface of normal cells;
2. Molecules present uniquely on the surface of cancer cells.

Thus the remarkable specificity of antibodies makes them promising agents for human therapy, for example:

1. Being able to make an antibody that will bind only to the cancer cells in a patient
2. Coupling a cytotoxic agent (e.g., a strong radioactive isotope) to that antibody.
3. Giving the complex to the patient so it can seek out and destroy the cancer cells (but spares the normal cells).

What is the procedure?

Step 1: Spleen cells from a mouse that has been immunised by a desired antigen are mixed with myeloma cells.

For successful fusion of these two cells, the myeloma cells:

A): Should have lost the ability to synthesise hypoxanthine-guanine-phosphoribosyl transferase (HGPRT, an enzyme which enables cells to produce purines with the help of extracellular hypoxanthine as precursor). Ordinarily, the absence of HGPRT is not a problem for the cell because cells have an alternate pathway that they can use to synthesise purines. However, when cells are exposed to aminopterin (a folic acid analogue), they are unable to use this other pathway and are then fully dependent on HGPRT for survival;

B): Must have lost the ability to synthesise any antibody molecules of their own (so as not to produce a hybridoma producing two kinds of antibody molecules).

The first property is exploited by transferring the cell fusion mixture to a culture medium called HAT medium because it contains:

- hypoxanthine
- aminopterin
- the pyrimidine thymidine

What is the logic behind this?

- Unfused myeloma cells cannot grow because they lack HGPRT.
- Unfused normal spleen cells cannot grow indefinitely because of their limited life span.
- Hybridoma cells (produced by successful fusions) are able to grow indefinitely because the spleen cell partner supplies HGPRT and the myeloma partner is immortal.

Step 2: Test the supernatants from each culture to find those producing the desired antibody.

Step 3: Because the original cultures may have been started with more than one hybridoma cell, you must now isolate single cells from each antibody-positive culture and subculture them.

Step 4: Again, test each supernatant for the desired antibodies. Each positive subculture – having been started from a single cell – represents a clone, and its antibodies are monoclonal. That is, each culture secretes a single kind of antibody molecule directed against a single determinant on a preselected antigen.

Step 5: Scale-up the size of the cultures of the successful clones.

Hybridoma cultures can be maintained indefinitely:

- *in vitro*; that is, in culture vessels. The yield runs from 10 – 60 µg/ml.
- *in vivo*; i.e., growing in mice. Here the antibody concentration in the serum and other body fluids can reach 1 – 10 mg/ml. However, animal welfare activists in Europe and in the USA are trying to limit the use of mice for the production of monoclonals.

What is recombinant mAB ?

The production of recombinant monoclonal antibodies involves technologies, referred to as *repertoire cloning* or *phage display*. Recombinant antibody engineering involves the use of viruses or yeast to create antibodies, rather than mice. These techniques, trying to limit the use of mice for the production of monoclonals, rely on rapid cloning of immunoglobulin gene segments to create libraries of antibodies with slightly different amino acid sequences from which antibodies with desired specificities can be selected[8]. These techniques can be used to enhance the specificity with which antibodies recognise antigens, their stability in various environmental conditions, their therapeutic efficacy, and their detectability in diagnostic applications[9]. Fermentation chambers have been used to produce these antibodies on a large scale.

Uses for monoclonal antibodies

Monoclonal antibodies are widely used as diagnostic and research reagents. Their introduction into human therapy has been much slower.

Diagnostic applications

1. Western blot test (to dectect a protein on a membrane)
2. Immunofluorescence test (to detect a substance in a cell)
3. Immunohistochemistry (to detect antigen in fixed tissue[10])
4. Immunoprecipitation and affinity chromatography (technique to purify a substance)

What are chimeric and humanised mABs ?

The main difficulty is that mouse antibodies are "seen" by the human immune system as foreign, and the human patient mounts an immune response against them, producing HAMA ("human anti-mouse antibodies"). These not only cause the therapeutic antibodies to be quickly

eliminated from the host, but also form immune complexes that cause damage to the kidneys.

Monoclonal antibodies raised in humans would lessen the problem, but few people would want to be immunised in an attempt to make them, and most of the attempts that have been made, have been unsuccessful.

Using genetic engineering it is possible to make mouse-human hybrid antibodies in an attempt to reduce the problem of HAMA.

- **Chimeric antibodies:** The antibody combines the antigen-binding parts (variable regions) of the mouse antibody with the effector parts (constant regions) of a human antibody. Infliximab, rituximab, and abciximab are examples.
- **Humanised antibodies:** The antibody combines only the amino acids responsible for making the antigen binding site (the hypervariable regions) of a mouse (or rat) antibody with the rest of a human antibody molecule thus replacing its own hypervariable regions. Daclizumab, Vitaxin, Herceptin, are examples[11].

In both cases, the new gene is expressed in mammalian cells grown in tissue culture (*E. coli* cannot add the sugars that are a necessary part of these glycoproteins). A solution to this problem would be to generate human antibodies directly from humans. However, this is not easy, primarily because it is generally not seen as ethical to challenge humans with antigen in order to produce antibody; the ethics of doing the same to non-humans is a matter of debate. Furthermore, it is not easy to generate human antibodies against human tissues. Another approach involves mice genetically engineered to produce more human-like antibodies. Monoclonal antibodies have been generated and approved to treat: cancer, cardiovascular disease, inflammatory diseases, macular degeneration, transplant rejection, multiple sclerosis, and viral infections.

Therapeutic applications

In immune-mediated diseases

1. **Muromonab-CD3 (OKT3)** and two humanised anti-CD3 monoclonals. Bind to the CD3 molecule on the surface of T-cells. Used to prevent acute rejection of an organ, e.g., kidney, transplants. The humanised versions show promise in inhibiting the autoimmune destruction of beta cells in type 1 diabetes mellitus[12].
2. **Infliximab:** Binds to tumour necrosis factor-alpha (TNF-α). It is effective in some inflammatory diseases such as rheumatoid arthritis (by blunting the activity of Th1 cells). Side-effects: can convert a latent case of tuberculosis into active disease; can induce the formation of autoantibodies (by promoting the development of Th2 cells).
3. **Omalizumab:** Binds to IgE, thus preventing IgE from binding to mast cells. Shows promise against allergic asthma.
4. **Daclizumab:** Binds to part of the IL-2 receptor produced at the surface of activated T-cells. Used to prevent acute rejection of transplanted kidneys. Has also shown promise against T-cell lymphoma.
5. **Adalimumab:** Inhibits tnf-alfa. Used in rheumatoid arthritis (RA). It is a humanised mAB.
6. **Etanercept:** TNF receptor. Used in RA.

In haematological malignancies and solid tumours

1. **Rituximab** binds to the CD20 molecule found on most B-cells and is used to treat B-cell lymphomas.
2. **Zevalin:** This is a monoclonal antibody against the CD20 molecule on B-cells (and lymphomas) conjugated to either:
   - the radioactive isotope indium-111 (^{111}In) or
   - the radioactive isotope yttrium-90 (^{90}Y)

   Both are given to the lymphoma patient, the ^{111}In version first followed by the ^{90}Y version (in each cases supplemented with RITUXIMAB).
3. **Tositumomab:** This is a conjugate of a monoclonal antibody against CD20 and the radioactive isotope iodine-131 (^{131}I). It, too, is designed as a treatment for lymphoma.

On 3rd February 2005, the *New England Journal of*

Medicine (NEJM) reported that 59% of patients with a B-cell lymphoma were disease-free 5 years after a single treatment with ^{131}I-tositumomab.

4. **Mylotarg:** A conjugate of a monoclonal antibody that binds CD33, a cell-surface molecule expressed by the cancerous cells in acute myelogenous leukaemia (AML) but not found on the normal stem cells needed to repopulate the bone marrow.

5. **LymphoCide:** Binds to CD22, a molecule found on some B-cell leukaemias.

6. **Alemtuzumab:** Binds to CD52, a molecule found on white blood cells. Has produced complete remission of chronic lymphocytic leukaemia.

7. **Lym-1:** Binds to the HLA-DR-encoded histocompatibility antigen that can be expressed at high levels on lymphoma cells.

8. **Gemtuzumab:** Used in relapsed AML.

9. **Trastuzumab:** Binds her2, a receptor for epidermal growth factor (EGF) that is found on some tumour cells (some breast cancers, lymphomas). So far, it is the only monoclonal that seems to be effective against solid tumours. Epithelium specific MAb to diagnose malignancy in serous effusions[13].

Angiogenesis inhibitors

1. **Vitaxin:** Binds to a vascular integrin (alpha-v/beta-3) found on the blood vessels of tumours but not on the blood vessels supplying normal tissues. In Phase II clinical trials, vitaxin has shown some promise in shrinking solid tumours without harmful side-effects.

2. **Bevacizumab:** Binds to vascular endothelial growth factor (VEGF) preventing it from binding to its receptor. Approved by the US FDA in February 2004 for the treatment of colorectal cancers.

In cardiology

1. **Abciximab:** Inhibits the clumping of platelets by binding the receptors on their surface that normally are linked by fibrinogen. Helpful in preventing reclogging of the coronary arteries in patients who have undergone angioplasty.

2. MAb against cardiac myosin is used to detect and quantify myocardial cell death[14].

3. A small American company OrbusNeich has come up with the idea of developing a stent with a completely different mechanism of action. This stent, which is baptised "Genous", accelerates the repair of the vessel. To achieve this, the stent is coated with monoclonal antibodies capable of capturing the circulating precursors of endothelial cells, in charge of vessel wall repair. Hopes are that in this way inflammatory reactions and blood clot formation can be prevented.

   Antibodies, long used as discriminating tools in immunoassay, are now being used *in vivo*, both in diagnosis and therapy. In cardiovascular medicine, applications that have reached the stage of clinical trial include the reversal of digitalis intoxication by digoxin-specific antibodies and the imaging of cardiac necrosis with monoclonal myosin-specific antibodies. An exciting future prospect, still in an early experimental stage, is the application of fibrin-specific monoclonal antibodies to both the visualisation of thrombi and emboli and the targeting of fibrinolytic agents[15].

4. Monoclonal antibody technology has resulted in an entirely new class of agents, which have been applied to a variety of problems in cardiology. The four antibodies, which have been most widely used in clinical cardiology, are Digibind, OKT3, Myoscint, and 7E3. Each demonstrates the unique potential for the use of antibodies in clinical cardiology[16].

5. The major value of hot-spot imaging of the myocardium is its ability to define areas of necrosis rather than areas of diminished blood flow or cellular function. Applications of hot-spot imaging include the diagnosis and quantitation of myocardial infarction, myocarditis, and cardiac transplant rejection. The two agents in clinical use, 99mTc-Pyrophosphate and radiolabelled antimyosin, are discussed.

Monoclonal antibodies in imaging

Over the past year, the FDA advisory committees

recommended market approval of four radiolabelled antibodies for diagnostic imaging. At this time, the agency has granted a license to three of the manufacturers to market their imaging agents.

1. **CEA-Scan** is a murine monoclonal antibody fragment linked to technetium 99m. CEA-Scan is reactive with carcinoembryonic antigen, a tumour marker for cancer of the colon and rectum. It is indicated with other standard diagnostic modalities for the detection of recurrent and/or metastatic colorectal cancer. In conjunction with computed tomography, the agent provides additional information about the presence, location, and extent of disease. CEA-Scan was approved June 26, 1996.

2. **Myoscint** is a murine monoclonal antibody Fab fragment linked to indium-111. Indium-111 Myoscint binds with high affinity and specificity to human cardiac myosin, which is exposed following a loss of integrity of the myocyte cell membrane. It is a cardiac imaging agent indicated for detecting the presence and location of myocardial injury in patients with suspected myocardial infarction. Use of Myoscint is anticipated in situations where electrocardiography and cardiac enzymes are nondiagnostic. Myoscint was approved July 3, 1996.

3. **Verluma** is a monoclonal antibody Fab fragment linked to technetium-99m. Verluma identifies advanced stage disease in patients with small cell lung cancer (SCLC). The determination of disease stage has important prognostic and therapeutic implications. Verluma was approved August 20, 1996.

4. **ProstaScint** is a monoclonal antibody imaging agent linked to indium-111. It seeks out and attaches to prostate cancer and its metastases. ProstaScint images can aid in patient management by helping identify when the cancer has spread outside the prostatic bed to regional lymph nodes or to distant soft-tissue sites. Surgical resection of the prostate is not indicated for patients whose disease has spread outside the prostatic bed.

In neurology

1. **Natalizumab:** A monoclonal antibody against alfa-4 integrin has been approved by FDA for treatment of multiple sclerosis[17].

2. Researches are also on way to use MAb to promote remyelination in chronic demyelinating neurologic disorders[18].

3. Monoclonal antibodies directed against adhesion molecules participating in PMNL-endothelium adherence are capable of attenuating cerebral vasospasm after SAH. These findings provide the first evidence for a role of the ICAM-1/LFA-1 interaction in the pathogenesis of cerebral vasospasm. Although these findings are preliminary, they provide encouragement that inhibitors of cellular inflammation may prove a novel clinical avenue for the treatment of cerebral vasospasm[19].

Conclusion

Infinity cannot be concluded. But to still follow the protocol, monoclonal antibodies since the time of their invention have revolutionised diagnostic and therapeutics in clinical medicine. And it will not be premature to state that they may write a whole new chapter in modern medicine in the near future.

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DIAMICRON

REVIEW ARTICLE

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Acute renal failure in the elderly

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Abstract

The aim of this review is to highlight the emerging phenomenon of increasing incidence of acute kidney injury (AKI)/ acute renal failure (ARF) in the elderly population worldwide. With increase in longevity, proportion of elderly men and women suffering from ARF in hospitals has risen. Newer nephrotoxic drugs, multiple invasive procedures, avoidable radiocontrast studies in modern hospitals have contributed to this phenomenon. Age related decline in glomerular filtration rate (GFR) make the elderly and very old vulnerable. ARF is a poor prognostic marker in critically ill patients. Increased relative risk of comorbid factors has been confirmed from recent studies. Furthermore, demonstration of molecular determinants of acute kidney injury has helped in understanding the initiating stage better. Although age-related reduction in GFR is unavoidable, and advanced molecular treatment to prevent AKI is for the future, physicians, surgeons and general practitioners will do well exercising caution in avoiding nephrotoxic drugs, unnecessary radiocontrast studies, and better fluid and electrolyte management that will significantly reduce incidence of AKI/ ARF in the elderly.

Key words: AKI/ARF, elderly, vulnerability, rising incidence, ICU, sepsis, contrast toxicity, preventability.

Introduction

Acute renal failure (ARF) is currently undergoing conceptual change to be better termed as acute kidney injury (AKI)[1]. The change in terminology was felt necessary due to the following reasons: (i) Recognition that even relatively mild changes in serum creatinine are associated with adverse outcome in hospitalised patients. Thus the syndrome should encompass more than just kidney failure alone; (ii) Realisation that the term 'injury' conveys pathophysiology and pathogenesis more accurately than the term failure; (iii) The word kidney is more readily understood by the people than 'renal'. Recent studies have revealed urinary and blood biomarkers that may help in detecting early, AKI, before a rise in serum creatinine. However, despite detection of several biomarkers which may create a new paradigm in the definition of AKI, to date these biomarkers have not been validated in multiple heterogeneous populations.

Therefore, for the present, the definition and classification of AKI remain the same as that of ARF which is defined as rapid deterioration (i.e., over hours to weeks) of renal function (potentially reversible) resulting in accumulation of nitrogenous waste products in the body and which are not due to pre-renal factors. However, it is important to remember some of these pre-renal factors if left untreated may progress to established acute tubular necrosis (ATN). Renal ischaemia induced by loss of body fluid, hypotension, or toxins, is classically and commonly implied in pathogenesis of ATN.

ARF occurs in approximately 5% of all hospitalised patients[2], and this incidence is likely to rise further with the introduction of more nephrotoxic drugs, more complex invasive procedures and as world witnesses more physical trauma due to accidents, terrorist attacks, or natural disasters like earthquakes. Patients above 70 yrs of age have ARF 3.5 times more common than the younger patients[3].

Susceptibility to ARF in the elderly is due to profound changes taking place in structure and function of kidneys with age. Greater proportion of elderly people are now seen occupying hospital beds with renal disorders, many of whom are diagnosed as a part of hospital acquired renal failure. Common systemic diseases observed in the elderly make them vulnerable to develop AKI. Some conditions occuring frequently in the elderly such as cancer, coronary artery disease, myeloma may also complicate acute renal failure either by direct involvement or by investigational procedures, operations conducted, or therapeutic agents given to patients.

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Pathophysiology of acute renal failure in general

Renal structures involved in pathogenesis of AKI are (i) Renal tubules which on histology may show acute tubular necrosis; (ii) Renal cortex (acute cortical necrosis); (iii) Glomeruli (acute glomerulonephritis, rapidly progressive glomerulonephritis, necrotising crescentic glomerulonephritis, haemolytic uraemic syndrome and so on); (iv) Interstitium (acute interstitial nephritis); (v) Renal vessels (at micro and macro level), renal vasculitis, widespread occlusion by fibrin thrombi or cholesterol emboli, besides there may be main arterial thrombo-embolism or venous thrombosis; (vi) Ureters, renal pelvis and collecting ducts (obstruction by stones, pigments, crystals, necrosed renal papilla, etc.) (Fig. 1).

Thus it is important to realise that ARF is not one disease, it is a syndrome caused by medical, surgical and gynaecologic disorders, lesions afflicting different locations of the renal system and mediated through different complex pathogenetic mechanisms. Although, literally, ARF may occur due to any one of the afore-mentioned structures being involved, conventionally ARF/AKI implies ATN and/or acute interstitial nephritis (AIN) due to ischaemic or toxic exposure.

Clinical features and presentations are different from patient to patient and so is the prognosis. For instance, prognosis of patients with high-risk ARF (Table I) with multi-organ system failure (MOSF) and hypercatabolic state can be much more grave than a simple non-oliguric drug-induced mild acute renal failure. Proper management therefore needs understanding of pathogenesis and identification of aetiologic factors.

Epidemiological profile

Community acquired ARF may affect the elderly population in tropical countries due to diarrhoeal illness, pneumonia, and other infectious diseases like malaria. Animal toxins[4] (snake bite, hornet bite) and plant toxins, alternative medical therapies, continue to cause AKI in some parts of the world. Natural and unnatural disasters like earthquakes, road accidents, and gunshot injury are on the rise globally, causing crush syndrome which contributes to high-risk ARF.

Hospital acquired ARF involving elderly population is on the rise due to increased use of nephrotoxic drugs (antibiotics, non-steroidal anti-inflammatory drugs (NSAID), ACE-inhibitors or angiotensin receptor blockers (ARB), anti-cancer and anti-viral therapies). One important

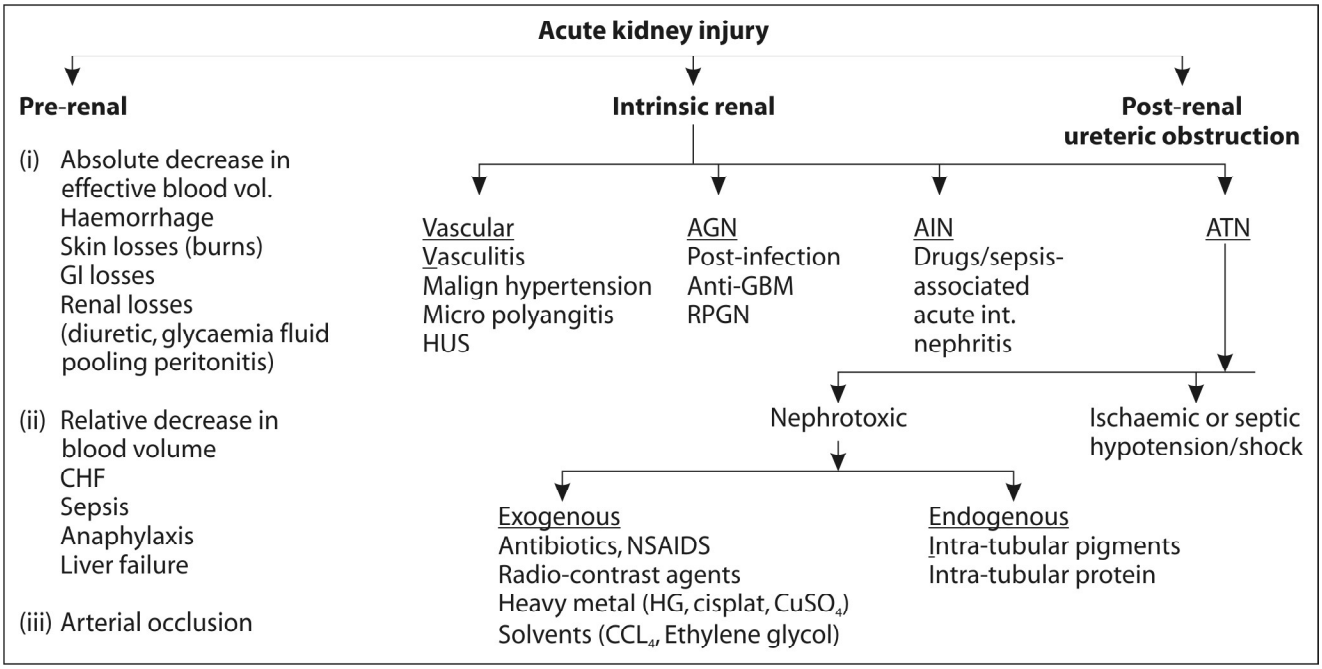

Fig. 1: *Acute kidney injury.*

cause is radio-contrast used for coronary angiogram in elderly diabetics, infection acquired through various intravenous and central lines inserted in sick elderly patients. A large study on AKI (from the US) showed incidence of mortality as high as 23 – 79%[5] in the ICU, and the majority were elderly. A registry of critically ill ARF patients to improve their care, has recently developed models for prognostic stratification and risk adjustment for mortality. The model included factors such as old age, sepsis, CHF, CNS failure, liver failure, haemotologic failure and dialysis status. This model clearly demonstrated relative risk of each co-morbidity when associated[6].

Critical mechanisms of ischaemic renal tubular cell injury

Complexity of *in vivo* studies involving continually changing variables make the interpretation of ischaemic tubular epithelial cell injury in ATN difficult. Therefore attempts have been made to utilise cell culture studies to elucidate many cellular alterations that occur in sub-lethal injury. These studies may allow future novel and specific therapies to minimise tubular cell injury.

During ischaemia, ATP depletion results in injury to proximal tubular cells that leads to dysregulation of actin cytoskeleton with loss of apical microvilli, membrance blebbing, redistribution of Na^+/K^+ ATP-ase, β1 integrins and other proteins with loss of cell polarity; disruption of tight junctions with opening of paracellular spaces and detachment of cell-cell and cell-substrate adhesion Membrane "blebs", apoptotic necrosed cells are shed which can obstruct tubular lumen causing increased intratubular pressure and decreased GFR. During recovery phase, polarity of injured cells is re-established and regeneration of tubular epithelium occurs (Fig. 2).

Loss of epithelial cell polarity results in re-distribution of proteins such as Na^+/K^+ ATP-ase, which in turn results in decreased proximal Na^+ re-absorption[7]. This leads to increased delivery of Na^+ to macula densa. To stop loss of massive amount of sodium and water from the body, afferent arteriolar vasoconstriction occurs under the effect of local renin angiotensin system at macula densa, which is known as tubuloglomerular feedback (TGF) causing near cessation of GFR. Actin dysregulation also results in loss of apical membrane ultra structure and detachment of cells from the basement membrane. Membrane 'blebs' and sloughed tubular cells cause tubular obstruction. Disruption of actin cytoskeleton results in disruption of tight junction proteins causing

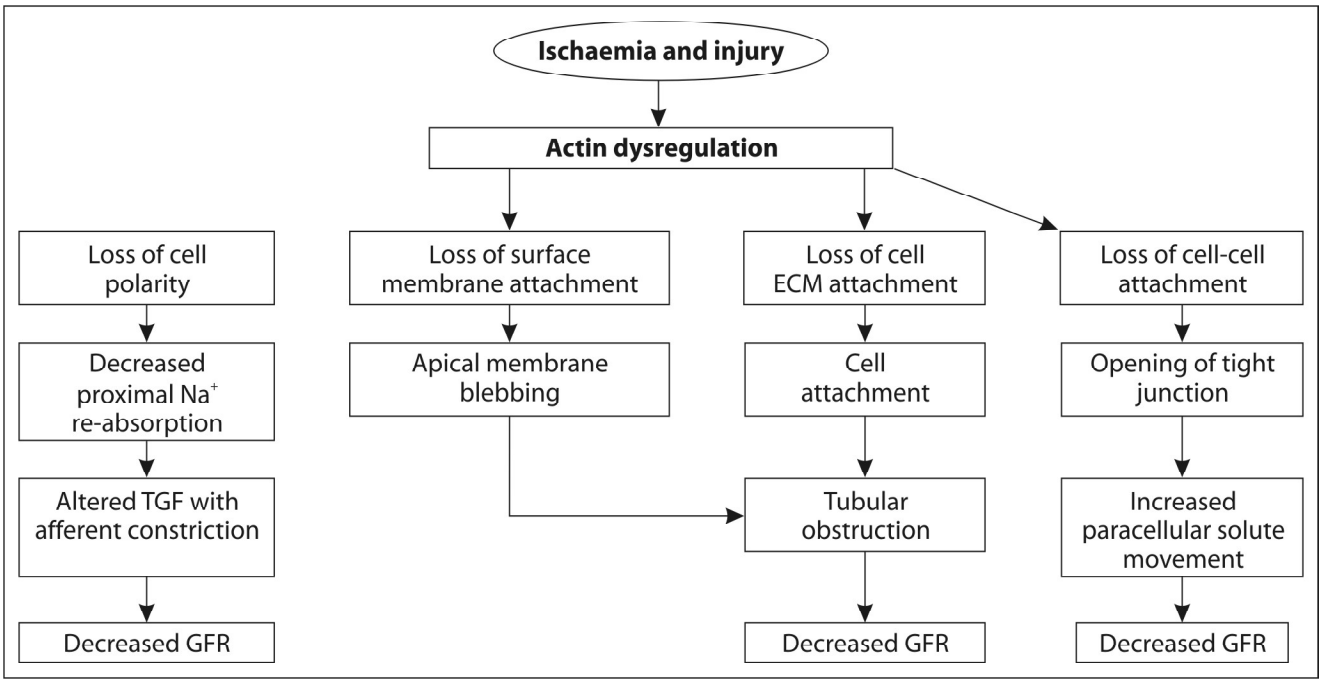

Fig. 2: *Renal ischaemia and injury.*

'back leak' of glomerular filtrate.

Pre-renal azotaemia

Vomiting, diarrhoea, excessive diuretic use cause pre-renal azotaemia frequently and faster in the elderly due to the age-related decrease in GFR. In addition, there is a failure of auto-regulation of RPF and impaired renal functional reserve which together make kidneys more susceptible to renal failure[3, 8].

Renal vasoregulatory mechanisms can be compromised with the use of NSAIDs, ACE-inhibitors, angiotensin II antagonists and alpha-adrenergic blockers. These drugs commonly used for rheumatic, cardiovascular and urinary obstructive conditions in the elderly may produce severe haemodynamic alterations leading to potentially reversible ARF. Low cardiac output, drug and toxin-induced impaired renal perfusion frequently cause pre-renal azotaemia. Similarly, septicaemia arising out of lung infections, cellulitits, gluteal abscess, gangrene in elderly diabetics are common causes of pre-renal azotaemia. Most such cases of ARF occur in hospital settings. Thus, greater awareness among physicians, surgeons, and gynaecologists is imperative in order to prevent as well as effectively treat pre-renal uraemia.

A recent study shows 60 per cent of elderly patients with pre-renal uraemia recover[8]. However, in the absence of prompt treatment it may evolve into established acute renal failure (acute tubular necrosis) in 23% in the elderly, compared to 15% in the younger patients[9]. Urinary index like fractional excretion of sodium (FENa) is unreliable in the elderly as increased FENa may occur in presence of hypo-perfusion due to pre-existing tubular defect[10], hence should be interpreted with caution.

Acute tubular necrosis (AIN)

ATN may result either due to severe acute ischaemic or toxic injury to renal tubules or when pre-renal factors continue to operate without prompt treatment or due to delayed treatment. Infection and septicaemia in elderly, more often in diabetics, is a major concern in hospitalised patients. Intubation, central lines, urinary catheter and at times diagnostic and surgical procedures are major contributors to infection. A variety of other infective lesions such as pneumonia, spontaneous bacterial peritonitis, cellulitis, gluteal abscess, infected gangrene, and occasionally internal organ abscesses also may cause septicaemia. Septicaemia especially due to Gram-negative organisms is more dangerous and often hospital acquired. It may complicate genito-urinary and hepatobiliary surgery. Gram-negative septicaemia leads to cytokine-driven inflammations and its injurious consequences more commonly as already described. It leads to endotoxin-induced renovascular vasoconstriction in about 30 per cent of elderly patients and intrarenal microvascular coagulation (DIC) ischaemia to kidneys as well as to other vital organs. Thus ATN develops in susceptible individuals who may even develop MOSF. Clearly, elderly patients in the ICU are at high-risk. They are haemodynamically unstable and usually receive nephrotoxic antibiotics such as aminoglycosides, anti-fungal drugs. Hence careful dose calculations and monitoring of blood levels are necessary. ACE-inhibitors or angiotensin-II antagonist therapy in saline-depleted elderly patients can cause AKI. Radiocontrast studies for aortic aneurysm, atherosclerotic renal artery stenosis carries risk of contrast-induced nephropathy and ARF. Occasionally it may complicate cholesterol embolism leading to irreversible ARF. In inexperienced hands the risk of dislodgement of atherosclerotic plaque during angiography has been reported.

Old age is a risk factor for radiocontrast nephropathy in addition to other risk factors such as renal insufficiency, diabetes mellitus, congestive heart failure, volume depletion, multiple myeloma, and high-dose contrast studies (>125 ml). Elderly diabetics with renal insufficiency develop nephrotoxic ARF in more than 38 per cent. Pathophysiology of ARF is not entirely clear in contrast-induced nephrotoxicity, though acute and prolonged vasoconstriction from radiocontrast infusion has been demonstrated in the elderly[11].

Acute interstitial nephritis (AIN)

A variety of drugs like cephalosporins, rifampicin, ciprofloxacin, sulphonamides, NSAIDs, over-zealous loop diuretic therapy, allopurinol, cimetidine may cause AIN in elderly patients as much as it does in younger individuals. Similarly, various infections with streptococci

or staphylococci, HIV, cytomegalovirus, and legionella disease may cause acute interstitial inflammation[12]. Activation of degradative enzymes damage the basement membrane and affect regeneration of renal tubular segment. GFR is reduced due to loss of functioning nephrons and inability of surviving nephrons to compensate with hyper-filtration.

Renal vascular disease

Rapidly progressive necrotising crescentic glomerulonephritis is the commonest form of acute glomerulonephritis to cause ARF in the elderly. Histologically, there is acute necrosis in the glomerulus with or withour crescents. Eight per cent of those were ANCA positive in one series[13]. Another study showed pauci-immune crescentic glomerulonephritis in 31.2% of 259 biopsies for ARF in patients 60-year-old or above[14]. In our series however, in 189 biopsy-proven RPGN patients only 3.1% were older than 55 years of age[9]. The risk of treatment with pulse corticosteroids, cyclophosphamide or plasmapheresis of RPGN in the elderly remains high. In general, the prognosis is poor at this age despite some successes claimed by uncontrolled series.

Incidence, spectrum, and predictors of mortality: a global report

In recent years, innumerable publications have highlighted advancing age as an important risk factor and many other associated risk factors already outlined.

In a study involving 2,722 patients of ARF 1,528 had mild-to-moderate renal failure. It was observed that old age and male gender were common factors in patients of mild-to-severe renal failure at admission associated with a variety of co-morbidities such as diabetes, ischaemic heart disease, hypertension and congestive heart failure[15]. Serum creatinine more than 3 mg/dl was significantly associated with higher mortality rate (50%) attributable to bacteraemia compared to those who had serum creatinine less than 3 mg/dl on admission.

Acute renal failure in the elderly population in India has been analysed in recent reports. It was observed that ARF among elderly is a common problem in renal practice and is responsible for 48.9% of admissions to the nephrology ward and consultations. Sepsis contributed to ARF in 75.4%, 57.9% needed dialytic support, 57.9% were critically ill with multi-organ failure. Acute interstitial nephritis manifesting as rapidly progressive renal failure (RPRF) was seen in 33.3% patients followed by vasculitis (23.3%)[16]. Myeloma cast nephropathy was seen in 20% of RPRF. Though ARF complicated only 1.6% of hospitalised elderly patients, it was associated with a high mortality rate of 61%. On multivariate analysis, critical illness, age more than 60 years, and sepsis were found to be independent predictors of mortality[17].

An Argentinian report on ARF in the elderly observed multifactorial origin and atypical presentation like the 'Intermediate Syndrome', which combined features of pre-renal azotemia and ATN[18] in clinical examination, and laboratory (blood and urine) parameters were misleading. The authors argued that prophylactic avoidance of nephrotoxic drugs remains the best option combined with adequate hydration. Dialysis treatment was found beneficial irrespective of age and carried a good prognosis. On the other hand, a multi-national study has suggested that critically ill ICU patients of ARF requiring renal replacement therapy were associated with high mortality rate[19]. Cholesterol crystal embolism (CCE) causing ARF has been described as a complication of atherosclerosis following angiography and vascular surgery[20]. Since prognosis of this condition is very poor, Japanese authors have treated these patients with low-dose prednisolone with good outcome, probably through amelioration of inflammatory reaction surrounding affected renal vessels[21].

Prevention and treatment

A great deal of information in preventing and limiting the extent of ARF has accumulated over the years. In each case, the risk and benefit ratio of any contrast study must be analysed. If the study is absolutely essential, hydration by hyposmolar saline before contrast administration has shown to have prevented AKI significantly. Similarly, caution must be exercised in elderly people before conducting complex diagnostic and therapeutic procedures. Nephrotoxic drugs should be avoided as much as possible.

Fluid balance in acute kidney injury

Recently, the deleterious effect of overzealous fluid therapy has been recognised. Saline overload can predispose to organ dysfunction, impaired wound healing, and nosocomial infection. It is now known that frequent fluid challenges in an oligoanuric patient produce more interstitial oedema, increase intra-abdominal pressure and delay renal recovery. Thus, a conservative fluid management has been found to be effective in large randomised control trials. Hypovolaemia and renal hypoperfusion can occur in patients of AKI if excessive removal by use of diuretics or by dialysis treatment. Thus accurate assessment of fluid status and fluid requirement targets need to be defined on a daily basis for improved outcome.

Dialysis

Several issues in dialytic therapy in ARF are currently being debated. These include the effect of intermittent versus continuous renal replacement therapy, improved biocompatible membrane on patient survival, optimal dialysis dose, and the benefit of removal of inflammatory mediators. Despite these controversies, use of biocompatible membrane is justified in critically ill patients. Similarly, CRRT offers advantages of better tolerance to dialysis, better volume control, increased delivered dose of dialysis, aggressive nutritional support, and possible anti-endotoxaemic and anti-inflammatory effects.

Conclusion

Elderly population is vulnerable to develop acute renal failure due to an age-related decline in GFR and RPF. Recent studies have refined our understanding of the aetiopathogenesis and natural history of ARF and ability to identify the risk of renal dysfunction early, particularly in hospitals and in ICUs. Presence of AKI causes exacerbation of impaired function of other vital organs in high-risk patients. Clearly, the term acute kidney injury should be preferred, although biomarkers to identify AKI early before rise in serum creatinine remain to be validated.

Incidence of ARF is rising in all countries due to newer nephrotoxic drugs, multiple hospital diagnostic and therapeutic procedures, and also due to a rise in natural (road accidents) and unnatural disasters (terrorism and earthquakes). Rising prevalence of diabetes is a key factor furthering the vulnerability of the middle-aged and elderly people to develop ARF. Recent studies have enhanced our insight into derangements in the metabolic milieu in a critically ill patient due to loss of kidney homoeostatic function. Arguably, the pathways involved are future potential therapeutic targets to improve prognosis of the patient. However, a large part of ARF in the elderly is preventable by simple avoidance of nephrotoxic drugs and substances, proper management of fluid and electrolytes and exercising caution in conducting avoidable procedures. In the developing countries, general practitioners, physicians, surgeons in commercially motivated hospitals need to be regularly educated through CMEs about these measures to make prevention of AKI successful.

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REVIEW ARTICLE

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Pivotal role of heart rate in health and disease

Trinath Kumar Mishra, Prabeen Kumar Rath***

Abstract

Since time immemorial, the pulse has been considered as a 'window' into the heart. Heart rate is one of the most important parameters measured from the pulse, and has aptly been referred to as one of the vital parameters. Heart rate is often influenced by physiological factors like the circardian cycle, posture, blood pressure, and physical activity. High heart rate is both causative and indicative of important pathophysiological processes. Heart rate might have deleterious effects on the process of atherosclerosis, degree and severity of ischaemia, and eventually heart failure. Resting heart rate has been directly related to all-cause mortality, cardiovascular mortality, and development of cardiovascular disease in the general population, and in patients with coronary artery disease. Thus, heart rate is an important therapeutic target. The new drug ivabradine, solely lowers heart rate by inhibiting I_f current of sino-atrial node and thus holds considerable promise for the future.

***Key words:** Heart rate, cardiovascular mortality, ivabradine.*

Introduction

Heart rate is (HR) is a predictor of major cardiovascular events in both the general population and patients with various cardiovascular diseases. The total number of heart beats in a lifetime remain fairly constant across species and there exists an inverse relationship between resting heart rate and life expectancy[1]. The association between resting HR and mortality has also been observed in patients with hypertension, metabolic syndrome, and the elderly[2]. Because of the importance of HR, the pulse has been considered as a "window" into the heart since time immemorial. It is therefore ironical that despite having been measured for hundreds of years, a thorough understanding regarding the pivotal role of heart rate in clinical practice is still lacking. In the present article, we shall review methods of HR measurement, different parameters influencing HR, its role in cardiovascular morbidity and mortality, and impact of HR reduction by drug therapy.

Methods of measurement of HR

Sources of variability are frequent for HR because its measurement is affected by a number of confounders. These include physical factors, psychic stimuli, environmental factors, body position, and methods of measurement[3]. Because of these reasons, the Consensus Panel of the European Society of Hypertension has recommended that sufficient information should be provided while reporting HR data[4]. These include: (i) resting period before measurement; (ii) environmental conditions; (iii) method of measurement; (iv) number of measurements; (v) duration of measurement; (vi) body position; and (vii) nature of the observer (i.e., physician, nurse).

Care should be taken to standardise the conditions of measurement. Alcohol, nicotine, and coffee consumption should be avoided in the hours preceding measurement. Room temperature should be comfortable and background noise should be avoided. The patient is then asked to relax as much as possible and to refrain from talking during the procedure. At least 5 minutes of rest is needed to achieve stable haemodynamic conditions. HR should be measured over a 30 second period by pulse palpation. At least 2 measurements in the sitting position should be obtained. The sitting position is preferred because blood pressure (BP) is more frequently measured in that position, and HR can be taken at the end of each BP measurement (Table I).

Traditionally, HR is measured by pulse palpation. The pulse rate is measured by counting beats over a set period of time (preferably 30 seconds) and multiplying that number by 2 to get the number of beats per minute. The pulse rate can be measured at any point on the body where an artery is close to the surface, including most commonly the radial, carotid, brachial, and femoral artery. If stroke volume is subject to high variability and there is pulse

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deficit as in multiple extrasystoles and atrial fibrillation, HR should be measured directly by heart auscultation.

Table I: Procedures for heart rate measurement.

- The patient should be allowed to sit for at least 5 minutes in a quiet room at a comfortable temperature.
- Heart rate should be measured over a 30-second period by pulse palpation.
- At least two measurements in the sitting position should be taken.
- In a patient in whom orthostatic blood pressure measurement is performed, heart rate should be measured after each blood pressure reading.
- Results may vary according to whether heart rate is measured by a doctor, a nurse, or an automatic device.
- Patients performing their own blood pressure measurements should also collect heart rate data.

Normality interval for resting HR

While resting, the adult human heart beats at about 70 – 75 beats per minute (bpm)[5]. The infant rate of heart beat is around 130 – 150 bpm, the toddler's about 100 – 130 bpm, the older child's about 90 – 110 bpm, and the adolescent's about 80 – 100 bpm. Women generally have higher HR than men, and this difference ranges from 3 – 7 bpm. Although the effect of age on HR is not well established, most studies have shown that HR tends to decrease with age[2]. In multivariate regression analyses, HR has been shown to decrease by 0.13 bpm/year in adulthood[2].

The normal limits of resting heart rate are not yet established. The reference range in adults is normally between 60 bpm (rates below this are termed bradycardia) and 100 bpm (rates above this are termed tachycardia). This normality interval clearly does not apply to HR when considered as a risk factor, because HR levels higher than 85 bpm have been shown to imply a considerable increase in risk in many epidemiological studies[5]. It is therefore evident that the definition of tachycardia as HR greater than 100 bpm cannot be accepted for risk evaluation.

Physiological determinants of HR

The circardian cycle

The cardiovascular system is highly organised and displays a distinct diurnal pattern in many functional domains, as evidence by pronounced variations in HR. Studies in normal persons have shown nadir in HR between 3.00 and 5.00 AM in the middle of the sleep cycle, and prior to rapid rise associated with waking that is seen approximately between 6.00 and 8.00 AM[6]. These observations explain the apparent temporal clustering of cardiovascular ischaemic events, including sudden cardiac death, acute myocardial infarction, ventricular arrhythmias, and arterial embolism. Consistent with this inference, from 24-hour ambulatory ECG recordings in 111 patients with chronic stable angina and proven CAD, the number of ischaemic episodes and cumulative duration of ischaemia manifested circardian variation: highest values were observed between 8.00 and 10.00 AM[7]. The variation in ischaemic activity was associated with similar circardian variation in HR and myocardial oxygen requirements.

Influence of posture

Humans are unique in being capable of maintaining an erect posture for prolonged periods. Adaptation to this position is therefore critical and humans have evolved a range of mechanical as well as vascular compensatory mechanism to counter the influence of gravity. The immediate response in healthy young adults of going from a supine to standing position is a prompt 30 – 50% rise in HR, which peaks at about 8 – 15 seconds and then tapers downwards[8]. Following this phase, there is a stabilised response, which occurs 30 seconds to 20 minutes after assuming an erect posture. Age has a significant effect on postural changes in HR. The increase in HR is inversely related to age with increases amounting to 18.5 bpm at age 30 year and 9 bpm at age 80 years[9].

Influence of blood pressure

Several epidemiological and physiological studies have shown a significant correlation between HR and blood pressure. A nationwide survey conducted in Belgium among 5,027 men and 4,150 women using multivariate analysis showed that BP is strongly correlated to HR[10]. HR progressively increases with ascending quintiles of systolic and diastolic BP. However, HR is more strongly associated with systolic BP and this relationship is more apparent among males[10].

Based on these findings, it is not surprising to find that there are also strong links between increased HR and clinical hypertension. Presence of tachycardia is more frequent among hypertensive individuals than among normotensives[11]. Unaware hypertensive subjects (SBP > 140 mmHg or DBP > 90 mmHg) have higher probability of elevated resting HR and a higher mean resting HR (69.8 ± 9.8 bpm) than normotensive subjects (66.3 ± 8.6 bpm)[12].

Several potential possibilities have been raised to explain the mechanism underlying the close association between the resting HR and elevated BP. One possibility is that increased HR among hypertensive is an extension of the well described "white coat" phenomenon that interferes with assessment of BP. It is also plausible that both elevated BP and fast HR are epiphenomenon of a common defect underlying a complex clinical condition[2]. There is also support for a primary role of tachycardia in the association, as it is recognised that elevated HR increases heart work load and arterial shear stress as well as contributing to arterial stiffness[13].

Influence of physical activity

The increases in HR associated with dynamic exercise comprise parasympathetic withdrawal at low workloads and sympathetic stimulation as the predominant mechanism at high workloads. Withdrawal of parasympathetic stimulation causes a very rapid increase in HR, which peaks after only 10 – 20 seconds and is followed by a tendency towards stabilisation during the first minute of exercise. In contrast, sympathetic stimulation leads to a less dramatic linear increase in heart rate, which is detectable during the first 1 – 4 minutes of exercise.

With regular physical activity, which increases exercise tolerance over time, studies have shown that such conditioning lowers minimal HR during the day and night and allows the attainment of a higher maximal HR during exercise stress testing[14]. For subjects, who walked > 1 hour per day, the mean morning HR was 65.7 ± 7.8 bpm, which was significantly lower than for those who walked for < 1 hour per day (67.8 ± 7.8 bpm, $p < 0.001$)[15].

Resting HR and cardiovascular diseases

Several epidemiological studies support resting HR as a predictor of total and cardiovascular mortality[16]. The Chicago Peoples Gas Company Study (including 1,233 men followed-up for 15 years), the Chicago Western Electric Company Study (including 1,899 men follow-up for 17 years), and the Chicago Heart Association Detection project (including 5,784 men followed-up for 5 years), reported together in 1980, were among the earliest to demonstrate the prognostic significance of resting HR for all cause mortality in large population[17]. Multivariate analysis using age, blood pressure, total blood cholesterol, smoking and body weight as covariates found HR to be an independent predictor of both sudden cardiac death (SCD) and non-cardiovascular mortality in two of the three cohorts studied. The Framingham study demonstrated a significant relationship – in both men and women – between heart rate, cardiovascular mortality, coronary heart disease and sudden coronary death[18] (Fig. 1). In a French cohort, including 5,713 asymptomatic working men between the age of 42 and 53 at study entry, 23 years follow-up demonstrated a significant association between resting HR and both sudden and myocardial infarction-related death[19]. Resting HR > 75 bpm defined a relative risk of 3.92 for sudden death compared with HR < 60 bpm.

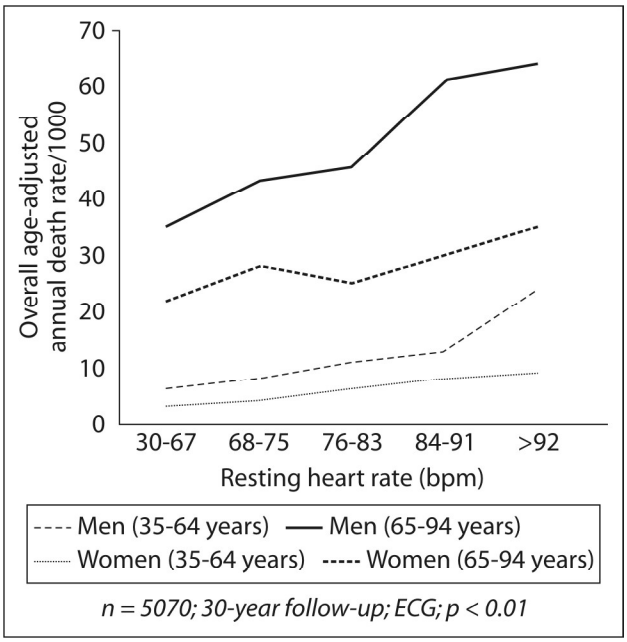

Fig. 1: *Resting heart rate and all-cause mortality in the Framingham Study*[18].

Resting HR in patients with coronary artery disease (CAD)

The prognostic value of resting HR has also been shown in patients with CAD. A total of 24,913 patients with

suspected or proven CAD from the Coronary Artery Surgery Study (CASS) registry were studied for a median follow-up of close to 15 years by Tardiff[20]. All-cause and cardiovascular mortality and cardiovascular re-hospitalisations were increased with increasing HR ($p < 0.0001$). When compared with the reference group, patients with resting HR ≥ 83 bpm at baseline had a significantly higher risk for total mortality [hazard ratio 1.32; confidence interval (CI) 1.19 – 1.47; $p < 0.0001$] and cardiovascular mortality (hazard ratio 1.31; CI 1.15 – 1.48; $p < 0.001$) after adjustment for multiple clinical variables.

In a study of 8,915 patients first seen when acutely ill with myocardial infarction, Zuanetti *et al* found that in-hospital mortality increased progressively with increasing HR (7.1% for HR < 60 bpm to 23.4% for HR > 100 bpm)[21]. HR was available at discharge in 7,831 patients in whom 6-month mortality was directly related to discharge HR (from 0.8% for HR < 60 bpm to 14.3% for HR > 100 bpm).

HR and pathophysiology of cardiovascular diseases

Local haemodynamic forces acting on the arterial wall include flow-generated shear stress, which is the tangential force due to the friction of the blood flowing on the endothelial surface and BP-derived tensile stress, also called circumferential stress, which represent the BP derived force imposed on the circumference of the arterial wall[22]. Tensile stress is sensed by mechanoreceptors which trigger a cascade of signalling molecules. Elevated tensile stress induces direct endothelial injury and increases endothelial permeability to low density lipoprotein (LDL) and to circulating inflammatory mediators[23]. Very high HR (> 120 bpm) by reducing diastolic phase reduces the stroke volume and cardiac output. Moderate tachycardia (close to 100 bpm) increases BP and the tensile stress and may promote endothelial injury and stiffness.

There is experimental and clinical evidence that suggests that sustained elevations in HR may play a direct role in the pathogenesis of coronary atherosclerosis[20]. HR was significantly correlated with the severity and progression of atherosclerosis on coronary angiography among men who had developed myocardial infarction (MI) at a young age[24]. Accelerated atherogenesis resulting from increased HR may be due to both mechanical and metabolic factors. An elevated HR is also associated with an increased risk of plaque rupture. Thus, HR may be involved in different phases of development of atherosclerosis, in plaque erosion, and plaque rupture resulting in thrombosis and in acute coronary event (Fig. 2).

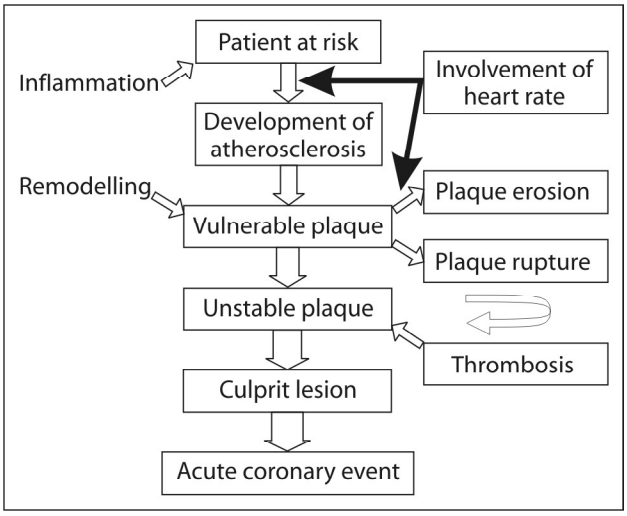

Fig. 2: *Role of heart rate in the development and progression of atherosclerosis.*

HR and myocardial ischaemia

Ischaemia results from imbalance between myocardial oxygen demand and supply (Fig. 3). Oxygen delivery to the heart mainly occurs during diastole, and the fraction of the cardiac cycle occupied by diastole increases as the HR decreases. Therefore, HR reduction improves diastolic perfusion time and myocardial perfusion. Furthermore, often under ischaemic conditions, stenotic coronary

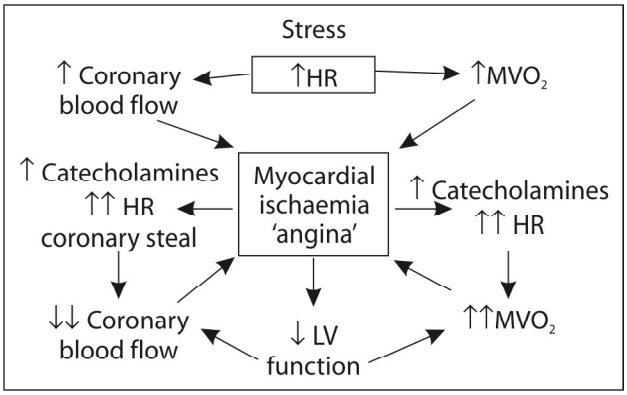

Fig. 3: *Mechanism of myocardial ischaemia as a mismatch between oxygen demand and oxygen supply by coronary blood flow.*

arteries are connected via collaterals to intact or less severely stenotic arteries[25]. This causes a typical redistribution of coronary blood flow with possible steal phenomenon. Any increase in HR would be deleterious, as it will further reduce diastolic perfusion, increase stealing from the ischaemic zone, and impair the flow at the ischaemic obstruction, which in turn, further compromises coronary flow[25].

In patients with stable CAD, most episodes of ambulatory or exercise-induced myocardial ischaemia are preceded by an increase in HR. The likelihood of developing ischaemia is related to baseline HR. The frequency of ischaemic episodes in patients with CAD is related to their mean HR: Patients with HR > 80 bpm experience ischaemia almost twice as often as those with HR < 70 bpm[26].

Pharmacological heart rate reduction

Beta-blockers have been shown to reduce total mortality and sudden cardiac death after myocardial infarction. At least part of these beneficial effects have been ascribed to their effect on HR[27]. A recent meta-regression of randomised clinical trials of beta-blockers and calcium channel blockers in post-myocardial infarction patients strongly suggests that the beneficial effect of these agents is proportionally related to resting HR reduction[28]. A statistically significant relationship was found between resting HR reduction and reduction in cardiac death, all cause death, sudden death, and non-fatal myocardial infarction recurrence. Each 10 bpm reduction in HR is estimated to reduce the relative risk of cardiac death by 30% (Fig. 4). This meta-regression of randomised clinical trials strongly suggests that resting HR reduction could be a major determinant of the clinical benefit in this study.

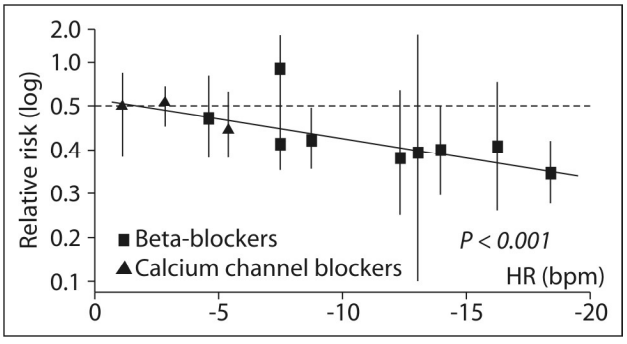

Fig. 4: *Heart rate lowering is associated with reduction in cardiac deaths in post-myocardial infarction patients[30].*

Recently, this hypothesis was tested within randomised controlled trials of beta-blockers in systolic heart failure. There was a close relation between all-cause annualised mortality rate and HR. A strong correlation between change in HR and change in ejection fraction was noted[29].

These data suggest that HR reduction is indeed a major component of the progressive benefit in post-MI and heart failure trials with beta-blockers and therefore should be an important therapeutic goal for the improvement of prognosis[23].

'Pure' heart rate reduction

Until relatively recently, pure HR slowing has not been possible. Available HR-slowing drugs (e.g., beta-blockers, certain calcium antagonists) also have other cardioactive properties, such as negative inotropy, vasoactivity that may be beneficial in some patients but unwanted in others, or may be responsible for adverse effects.

Ivabradine is a new medication, currently approved in Europe, that has HR-lowering properties without other direct cardiovascular effects. Ivabradine is the first of a new class of agents that act specifically on the sino-atrial node by inhibiting the I_f (funny current) current of cardiac pacemaker cells without affecting other cardiac ionic currents[23]. This drug has a unique pharmacodynamic profile as HR reduction is not associated with negative inotropic effects or vasodilatation.

Ivabradine has been investigated in patients with stable coronary artery disease. The drug has shown anti-anginal and anti-ischaemic efficacy when compared with well-established reference anti-anginal drugs, such as beta-blockers and calcium antagonists[31,32]. Whether the use of Ivabradine in patients with coronary artery disease and LV dysfunction results in the reduction of cardiovascular morbidity and mortality will be clarified by ongoing clinical trials like Beautiful study[23].

Summary and conclusions

Among various parameters which the ancient physicians used to measure from the pulse, one of the most important measures was heart rate. Since then, heart rate has been referred to as one of the "vital parameters". Hence, HR should always be measured meticulously, for

which the patient should be allowed to sit for at least 5 minutes in a quiet room at a comfortable temperature. HR should be measured over a 30 second period by pulse palpation and at least 2 measurements in the sitting position should be recorded. Heart rate is often influenced by several physiological determinants like the circardian cycle, posture, blood pressure, and physical activity.

Several observational studies, registers, and trials have identified HR as a risk marker for cardiovascular mortality, independent of other risk markers, including currently validated risk factors. Resting HR has been directly related to all cause mortality, cardiovascular mortality, and development of cardiovascular disease in the general population and in patients with CAD. HR might have several deleterious effects on the cardiovascular continuum, affecting atherosclerosis, degree, and severity of ischaemia, and eventually hart failure due to CAD.

Thus, HR is an important therapeutic target. A drug like ivabradine, whose sole property is to reduce HR, holds considerable promise for the future. Ongoing studies like Beautiful study will clarify whether ivabradine use results in the reduction of morbidity and mortality in high-risk patients with CAD and LV dysfunction.

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CLINICAL RESEARCH

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Pulsed electromagnetic field energy in the management of ischaemic heart disease and heart failure

BM Hegde, CV Krishnaswami***

Abstract

Introduction: A patient with intractable left heart failure due to extensive advanced coronary artery disease, who was resistant to conventional treatment, was treated with pulsed electromagnetic energy field treatment using a new device, Em Probe, an invention of Late Dr. Glen Gordon of USA, that delivers short pulses of electromagnetic energy to heart muscle cells. This stimulates three important proteins inside the cell which rejuvenates the mitochondria. We now manufacture the same in India. One of us has the patent (BM). It, BM pulse, is ready for use right now.

Case history: An 86-year-old man was admitted with acute heart failure (NYHA Classification stage IV) at St. Isobel Hospital, Chennai, India on the 16th December 2008. He had been on ACE-inhibitors, diuretics, isosorbide dinitrate, aspirin, and other symptomatic treatments. Surgeons were not ready to do bypass surgery and the patient refused any invasive methods of management. We therefore fitted the electromagnetic pulsed wave dispenser onto his chest over the precordium, using adhesive tape. The machine is the size of a cell phone and runs on a 9-volt battery. The patient improved remarkably and has since been discharged to an assisted care home. He manages his day-to-day routine with very low doses of diuretics on a regular basis. On review a month later and eight months later he was in NYHAC stage I.

Conclusions: To the best of our knowledge, this is the first such report of this sort of treatment and the results were very encouraging. Since then we have treated 159 patients with multiple pathologies where cell damage was the basic damage due to ischaemia, injury, or inflammation, like heart attacks, brain attacks, fractures, inflammatory lesions, etc., using the device. The results are being readied for publication as a series.

Key words: Em probe, electromagnetic energy, intractable heart failure.

Introduction

A patient with intractable left heart failure (NYHA classification stage IV) due to extensive advanced coronary artery disease, who was resistant to conventional treatment, was treated with pulsed electromagnetic energy field treatment using a new device that delivers short pulses of electromagnetic energy to heart muscle cells. This stimulates three important proteins inside the cell which rejuvenates the mitochondria. They are HSP 70 (heat shock protein 70) nitric oxide (NO) synthase, and VEGF 165 gene protein (which is angio-genetic).

Case presentation

An 85-year-old male non-smoker presented with acute-on-chronic intractable left heart failure on the 16th December 2008 at St. Isobel Hospital, Chennai, India. He had been on ACE-inhibitors, diuretics, isosorbide dinitrate, aspirin, and other symptomatic treatments, but the left ventricular failure persisted.

He was admitted to the intensive care unit (ICU) on a weekly basis. Nocturnal dyspnoea was a frequent reason for admission. Even the slightest exertion, such as going to the toilet, would bring on severe anginal chest pain. The cardiac surgeons were not ready to do bypass surgery and the patient too refused any surgical intervention including angioplasty.

The patient had been a diabetic for 34 years, which had been managed with diet, exercise, and oral sulphonylureas and insulin as and when needed. The diabetes was well under control on admission. He had had his prostate surgery 10 years previously. He had experienced an acute anteroseptal myocardial infarction in 2005 and was treated conservatively. Multiple coronary vessel blocks were revealed by an angiogram with low ejection fraction but he refused any interventional procedures. He had also had a retinal detachment in 1972, which was corrected

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successfully with surgery.

We therefore fitted the electromagnetic pulsed wave dispenser onto his chest over the precordium, using adhesive tape. The machine is the size of a cell phone and runs on a 9-volt battery. A small light indicates when the power is low and the battery needs changing.

Since this device has no known side-effects, it can be left in place as long as necessary. The only precautions to be taken are: 1) avoiding contact with water which might short circuit the machine, and 2) switching it off inside airport security scanners to prevent an alarm being triggered as explained below.

Every cell in the human body is like a supercomputer and can derive its energy needs from the sun's electromagnetic field (Schumann rings)[1,2,3]. Ischaemic cells, however, cannot do this. Em Probe can deliver the same energy in a concentrated dose at the site of damage, either in the heart or the brain. As the energy generates some heat locally, it is pulsed to deliver energy for a fraction of a second and then rest for the larger part of the second.

Physiologically, each cell works like a supercomputer. The hardware is what we see under the microscope in a stained specimen in the laboratory. The software is the energy system in the thousands of proteins in the cytoplasm. They form two energy systems: high- and low-energy systems. The low-energy system consists mainly of three proteins with their genes: HSP 70 (heat shock protein 70), nitric oxide (NO) synthase, and VEGF 165 protein[1,2]. These three proteins get their energy from the device and transduce the energy to be supplied to the high-energy system that runs the 'hardware'. It is the stimulation of the NO synthase that makes the device trigger airport security systems – the level of NO goes-up 1,000-fold when the device is on. Since NO is also the core of TNT-loaded explosive devices, the airport security system picks this up.

The device was fitted to the patient on the 16 December 2008 after examinations including an echo and an electrocardiogram. The cardiac enzymes were all normal. There was no acute coronary syndrome although the left ventricular ejection fraction was very low.

During the weeks before the pulsed electromagnetic field device was fitted, the patient was mostly bedridden (propped up at 45 degrees), with frequent visits to the ICU. He used a mobile oxygen unit at home and took large doses of diuretics daily. His appetite was very poor and he was listless and disinterested in his surroundings.

On discharge from the hospital, he walked to the car and climbed stairs (one flight) at home without discomfort. From the 17 to the 26 December he steadily improved, sitting up most of the time and hardly sleeping during the day. Paroxysmal nocturnal dyspnoea (PND) reduced remarkably, except for the occasional shortness of breath in early morning on one day.

He went to a music concert (his first love) for the first time in two years and sat there for two hours having walked up a steep gradient in the auditorium without any difficulty. PND had almost disappeared and he had no need for oxygen or extra diuretics. He has been steadily improving since then. He continues to be reasonably well to carry on his usual daily chores and attend music festivals without additional oxygen or diuretics.

After improvement, the patient has been staying at an assisted care home. He manages his day-to-day routine and uses the device as and when needed. He is on small doses of anti-failure drugs: frusemide 40 – 80 mg once daily, baby aspirin 75 mg, and isosorbide dinitrate 5 mg three times daily and sub-lingually, when needed. The ACE-inhibitors and beta-blockers have been stopped. The patient continued to be in reasonable good health at a recent review on 15 July 2009 and is reported to be doing well even now (March 2010) and has not needed any hospitalisation since we began treatment in December 2008. We will monitor the patient to assess the long-term outcome of this treatment. The Table I gives the details of the echo reports done at different times.

Discussion

This is part of an ongoing study of a new method of non-invasive treatment for ischaemic heart disease. The relationships between the Schumann Resonance with life on earth made the pioneer, Dr Glen Gordon, devise this simple electromagnetic energy device using a 9-volt battery some years ago[1,2,3]. The device was extensively tested and the US FDA permitted its use initially for muscle damage with remarkable success[4,5,6]. Our own experience

with this device is very encouraging. We present the report of the first patient who was successfully helped using the device. "Enhancement of conformational adaptation of (cell) proteins in response to ischaemia-reperfusion injury is the goal of homeostasis, which, in reflecting the second law of thermodynamics, mandates external energy to achieve increased order and complexity", writes Glen Gordon in his seminal paper on energy medicine"[1]. We have treated more than one hundred and fifty patients to date with similar success either for cardiac or brain infarcts as also a variety of other cell damage situations.

pleasure he did not have for a long time before the Em Probe therapy. Notable was the fact that he did not have a single ICU admission in the interim. The latter was a routine affair for him at least twice a week before this therapy. We have several such success stories in the last 8 months to report at a later date.

Conclusions

The patient responded remarkably well to the electromagnetic energy treatment for his intractable left

Table I: Comparative cardiac echo studies of the patient.

Sl. No.	Parameters	Pre PEMFE*		Post PEMFE*	
		10/05/2007	11/14/2008	12/17/2008	12/25/2008
1	LV function	Mildly dilated; Apex – dyskinetic Severe LV dysfunction LV apical aneurysm	Dilated LV with akinetic apex. Moderate LV dysfunction	Dilated LV Severe LV systolic dysfunction. LV apical aneurysm.	Dilated; Moderate-to-severe LV dysfunction. LV diastolic dysfunction. Hypokinesia of lower 2/3 of IVS, apical LV, apicoposterior LV due to CAD.
2	LV Ejection Fraction	31%	35%	35%	40%
3	Valves	Normal Trivial MR Trivial AR	Normal Sclerosed aortic valve.	Normal mitral annular calcification. Severe mitral regurgitation. Trivial aortic regurgitation.	Normal MR + Trivial Aortic valvular sclerosis. No AS or AR
4	Any other Remarks	Severe hypokinesia of lower 2/3 of IVS, apical posterior LV. Mild pulmonary hypertension	CAHD. Hypokinesia of IVS and anterior wall. Basal IVS and basal inferior wall are contracting well. No pericardial effusion.	CAHD. Severe hypokinesia of lower 2/3rd of IVS, anterior wall and apical segment. No pericardial effusion.	Hypokinetic contraction of lower IVS, apical, apical posterior LV. Mild pulmonary hypertension.

*PEMFE * Pulse electromagnetic field energy*

Follow-up

A review on the 18th August 2009 did show that this patient is doing reasonably well and has not deteriorated in the last 8 months. His clinical assessment was satisfactory. He attends to his daily chores and also attends to music festivals, his hobby, as and when he desires, a

heart failure due to coronary artery disease. To the best of our knowledge, this is the first report of this treatment success.

Abbreviations

HSP, heat shock protein; ICU, intensive care unit; NO, nitric

oxide; PND, paroxysmal nocturnal dyspnoea.

Consent

The patient has signed a consent letter.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

BMH initiated the treatment and wrote up the case report. CVK was the consultant in charge of the patient for his day-to-day care at St. Isobel Hospital. He prepared the echo chart (Table I) as well.

Acknowledgement

We are indebted to all our colleagues, both at the World Academy of Authentic Healing Sciences in Mangalore and "Friends of Health", our parent body led by Professor Rustum Roy at Pennsylvania State University, USA, and at St. Isobel Hospital, Chennai, India, where one of us (CVK) heads the Unit. We are also grateful to Late Glen Gordon and his wife for being generous in passing on the USA patent (free of charge) on this device (when Glen was still alive) to one of us (BMH) for further developing and using for research and patient management.

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JIACM 2011; 12(4): 308-10

Lung cyst – A diagnostic dilemma

*Gouranga Santra**

A 22-year-old male patient presented with high grade fever and cough for 2 weeks duration. He had scanty expectoration and mild shortness of breath for the same duration. He was non-diabetic and had no history of chest trauma or contact with any patient of tuberculosis. Sputum for AFB was negative in three consecutive samples. Blood sugar was normal. ELISA for HIV (I and II) was negative. After straight X-ray chest and aspiration of pus, he was misdiagnosed to have pyopneumothorax (Fig. 1). Intercostal tube drainage was given along with broad-spectrum antibiotics. Subsequent chest X-ray showed that the tube occupied a giant cyst in left lung in a rounded manner (Fig. 2). Repeat chest X-ray (Fig. 3) and CT scan of thorax (Fig. 4 and 5) showed a well-defined thin-walled giant solitary cystic space in the lower lobe of the left lung with no evidence of rupture. The case was finally diagnosed as an infected giant congenital lung cyst.

Lung cysts are defined as air-containing spaces in lung parenchyma surrounded by a relatively thin (≤4 mm) wall. It is useful to distinguish lung cysts from cavities (air-

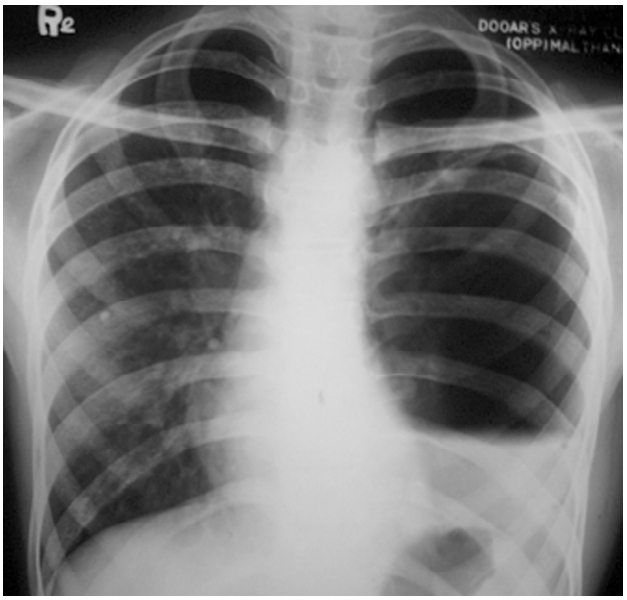

Fig.1: *Chest X-ray was misinterpreted as left-sided pyopneumothorax with horizontal air-fluid level. The left costophrenic angle appeared to be obliterated.*

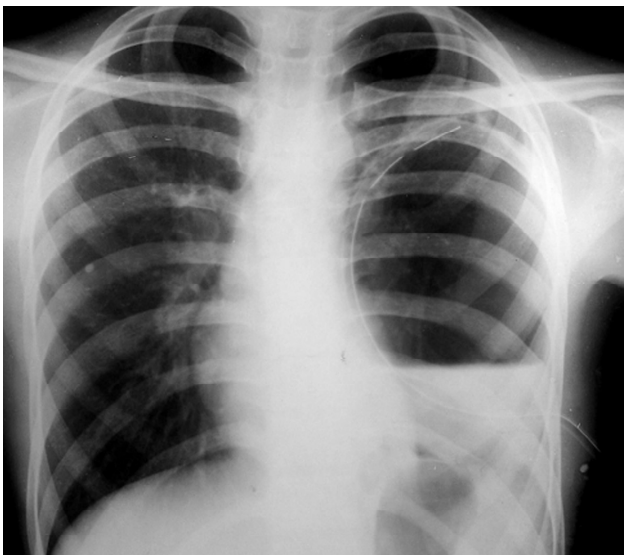

Fig. 2: *Chest X-ray showed the intercostal tube occupying a cyst in a rounded manner.*

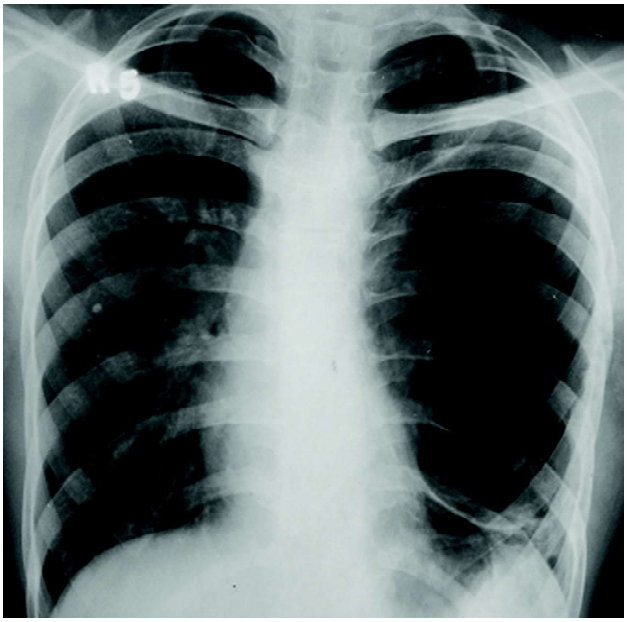

Fig. 3: *A thin-walled, well-defined and well-circumscribed cystic space occupied most of the left lung.*

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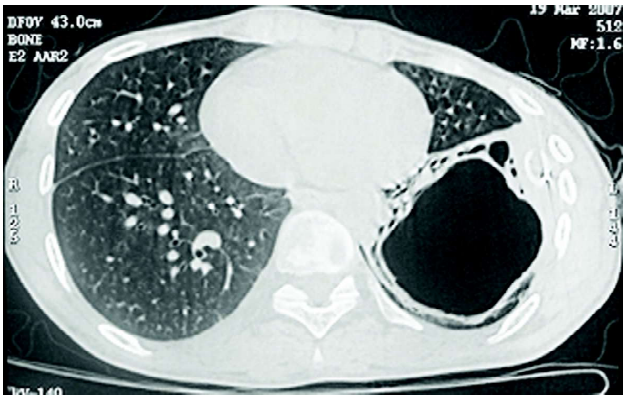

Fig. 4 and 5: *CT scan of thorax showed a well-defined, thin-walled cystic space, occupying the lower lobe of the left lung.*

containing lesions with a relatively thick > 4 mm, wall i.e., or within an area of a surrounding infiltrate or mass) and to categorise them as focal or multifocal from diffuse in distribution. Focal or multifocal cystic lesions in lung include congenital lung cysts, traumatic cysts, and infectious causes including coccidioidomycosis, pneumocystis carinii pneumonia, and hydatid disease. Malignant lesions rarely present as cystic lesions. Diffuse cystic disease is classically associated with pulmonary Langerhans cell histiocytosis (PLCH), lymphangioleiomyomatosis (LAM) and tuberous sclerosis.

Congenital parenchymal cysts (simple lung cysts) arise from any of the parenchymal tissues of the lung. The composition of the cyst wall is determined by its origin: bronchial glands, cartilage, or alveolar epithelium. Incidence and prevalence of lung cysts are not exactly known. Papagiannopoulos *et al* reported from their surgical experience in a small series where the simple cysts were the commonest cystic lung lesions in adults (40%)[1]. Simple cysts are unilocular and confined to a single lobe, commonly the lower lobe. Cysts may remain asymptomatic or enlarge resulting in respiratory distress. Cysts may also be infected. Chest X-ray is usually adequate for diagnosis. Infected giant lung cyst radiologically may resemble pyopneumothorax and should be assessed carefully to avoid misdiagnosis[2].

Other congenital cystic lung lesions are bronchogenic cysts, pulmonary sequestrations, and congenital cystic adenomatoid malformations (CCAM). A bronchogenic cyst is thought to develop as a diverticulum of the primitive foregut. Most are right-sided, midline, and in close proximity to the tracheobronchial tree. They can also migrate to the periphery. Approximately two-third of these are within the mediastinum, and one-third are intraparenchymal. They may contain normal tracheal tissue including mucus glands, elastic tissue, smooth muscle, and cartilage. Ciliated epithelium lines the cysts. Radiologically intra-pulmonary bronchogenic cysts are mostly opaque as they contain mucoid material and retained secretions. Rarely small patent bronchial communication is there and then the cyst appears air-filled. Bronchogenic cysts contain air-fluid level when infected. Sequestrations can be intra-lobar (anomalous parenchyma contained within visceral pleura), or extra-lobar (with a separate pleural covering). Sequestration appears as a persistent opacity or mass usually in the left lower lobe and contains air when infected. CCAM may present as a mass lesion with variable numbers of solid and cystic components. CCAM communicates with the bronchial tree and so contains air. CCAMs are often mistaken for congenital diaphragmatic hernia. Stocker classified three types of CCAMs: type I consists of large cysts, type II consists of small cysts, and type III shows homogeneous mass with cysts seen on microscopy[3]. These cysts are lined with ciliated columnar/pseudostratified columnar epithelium with mucous secreting cells. Absence of hyaline cartilage in the wall differentiates it from brochogenic cyst. In type I CCAM, a large dominant cyst is surrounded by smaller cysts; but when the cystic lesion is single, the differential diagnosis with the congenital parenchymal cysts and bronchogenic cysts may not be possible radiologically and therefore histopathologic examination is needed.

PLCH is a smoking-related diffuse lung disease affecting

males of 20 to 40 years age. The combination of satellite nodules, bizarre-shaped upper zone cysts, preservation of lung volume and sparing of costophrenic angles are characteristics of PLCH. Pulmonary LAM occurs in females of child-bearing age. Progressive proliferation of spindle cells (resembling smooth muscle cells) along the bronchioles leads to air trapping and development of thin-walled lung cysts. Rupture of these cysts can result in recurrent pneumothorax. Chylous pleural effusion may also be present. HRCT shows uniform thin-walled cysts in a diffuse distribution without zonal predominance. In tuberous sclerosis, lung disease appears similar to LAM but can also be seen in males.

True lung cysts need to be distinguished from emphysematous bullae (no visible walls), dilated airways (continuity and elongation of dilated airways on contiguous slices) and pneumatocoeles. Pulmonary pneumatocoeles can be single but more often are multiple, thin-walled, air-filled, cyst-like cavities. They may contain fluid levels. Initial chest X-ray often reveals pneumonia without evidence of pneumatocoele. Parapneumonic effusion or empyema can be present. Radiographic evidence of pneumatocoele occurs usually on fifth to seventh day of hospitalisation. Pneumatocoeles disappear within months after the pulmonary infection subsides.

Solitary congenital cysts can be treated with cystectomy or lobectomy if necessary. Lung cysts are often found to be CCAMs during surgery, though simple cysts do occur. Infected solitary cysts are treated with antibiotics and resected when quiescent. Multiple cysts are treated as part of underlying systemic disease. Surgical intervention is contraindicated for pneumatocoeles. Chest tube insertion is discouraged, as this can result in empyema.

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CASE REPORT

JIACM 2011; 12(4) : 311-3

Distal renal tubular acidosis presenting as periodic paralysis in a young female

Soumya Sarathi Mondal, Subhabrata Gangopadhyay**, Siddhartha Mani**, Debjoy Sau**, Rudrajit Paul**, Ramtanu Bandyopadhyay**

Abstract

A 17-year-old normotensive and non-diabetic female presented with acute onset flaccid paralysis with the history of a similar episode a few months back. Clinical and laboratory evaluation revealed lower motor neuron type of flaccid quadruparesis with hypokalaemia, normal anion gap metabolic acidosis, bicarbonaturia, and transtubular potassium concentration gradient (TTKG) more than 7. Subsequently urine acidification test (by NH4Cl challenge test) was done and diagnosis of distal renal tubular acidosis was established. The patient responded to conservative management (Sohl's solution).

***Key words:** Hypokalaemia, flaccid quadruparesis, urine acidification.*

Introduction

Distal-renal tubular acidosis (dRTA) is a non-uraemic syndrome of defective urinary acidification. It is characterised by presence of hypokalaemia, normal blood pressure, and normal anion gap metabolic acidosis, alkaline urine, inability to acidify urine pH < 5.5, nephrocalcinosis, and features of rickets. Primary dRTA can be inherited, but most cases are sporadic. An inherited case may be autosomal dominant or autosomal recessive form. Secondary causes are Sjögrens syndrome, amphotericin B toxicity, chronic active hepatitis, and SLE. The treatment required is alkali administration in the form of Sohl's solution in doses 0.5 to 2 ml/kg in 4 – 6 divided doses per day. We report a case of a 17-year-old female presenting with periodic acute onset flaccid quadruparesis. A diagnosis of d-RTA was established after a series of investigations.

Case report

A 17-year-old girl was admitted with acute onset quadruparesis evolving over a period of 24 hours and reaching upto the extent that she could only sit or stand with adequate support. She had a similar episode two months back which was preceded by diarrhoea and dehydration with hypokalaemia persisting even long after resolution of the diarrhoea. On assessment of the previous records, it revealed to be an episode of hypokalaemia leading to quadruparesis, recovered with oral KCl supplementation and discharged as hypokalaemic periodic paralysis with the advice to take oral KCl. But this time there was no history of diarrhoea. On examination, the only positive finding in the general survey was bradycardia (pulse 52/minute, regular). No features suggestive of thyrotoxicosis were present. Nervous system examination revealed normal higher functions, cranial nerves, sensory system, bladder and bowel, and cerebellar functions. Motor examination revealed normal muscle bulk with hypotonia. Muscle power of upper limb was 4/5 and lower limb was 3/5; deep tendon reflexes were present but diminished, and plantars were bilaterally flexor.

Investigation showed Hb – 11.2 gm%, TLC – 8,700/cmm (neutrophils – 72%, lymphocytes – 22%, eosinophils – 4%), MCH – 28.6 pg/cell, MCHC – 31.4 gm/dl, MCV – 91 fL. FBS was 108 mg/dl, CPK – 383 U/L, Na^+ – 134.8 mmol/l, K^+ – 2.29 mmol/l, Ca^{2+} – 3.8 mg/dl, Mg^{2+} – 2.8 mg/dl (normal – 1.5 to 2.6). ECG – prolonged PR interval, T-wave flattening and U wave. ABG performed: pH – 7.38, pO2 – 102 mmHg, pCO_2 – 20 mmHg, HCO_3 – 11.4 meq/l, Na^+ – 142 meq/l, K^+ – 2.6 meq/l, Ca^{2+} – 120.2 meq/l, anion gap – 10.4, plasma osmolality – 302.5 mOsm/kg. This ABG report showed a combination of metabolic acidosis with hypokalaemia. This condition is usually found in two possible conditions either due to GI loss or RTA. As there was no history or GI loss this time, we were strongly suspecting RTA.

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Other investigations included ANA – negative, fT_3 – 3.58 pg/ml, fT_4 – 7.2 mcg/dl, TSH – 1.59 IU/ml. 24 hours urine– total volume – 3,180 ml, K^+ excretion – 73.3 meq/24 hrs., i.e., 23.27 meq/l. Osmolality was 436.7 mOsm/kg and pH 7. Trans-tubular potassium gradient (TTKG) was 6.2. USG did not reveal any nephrocalcinosis. In the meantime, the patient was treated with oral potassium supplementation and patient dramatically improved. To confirm our diagnosis after stabilisation of the patient, we opted for an oral NH_4Cl challenge test. UTI was ruled-out beforehand by urine microscopy and culture. NH_4Cl was given orally at doses of 0.1 gm/kg with fruit juice.

Urine pH was subsequently recorded as follows:-

1st hour – 6.69

2nd hour – 6.19

3rd hour – 6.63

4th hour – 6.1

5th hour – 6.17

ABG – 2nd hour

pH – 7.29

HCO3 – 9.9 meq/l

Base excess – 14.5

Na^+ – 133 meq/l , K^+ – 2.4 meq/l

ABG – 4th hour

pH – 7.31

HCO_3 – 10.8 meq/l

Base excess – 13.7 mmol/l

Na^+ – 139 meq/l, K^+ – 2.4 meq/l, Cl – 118 mmol/l

Therefore, the urine pH did not decrease below 5.5 in spite of plasma HCO_3 being persistently below 20 meq/l. So our diagnosis of dRTA (type 1) was confirmed. Treatment with Sohl's solution (Na-citrate 500 mg, K- citrate 550 mg, and citric acid 334 mg/5 ml) was started. 1 ml of thin solution is equivalent to 1 meq of Na^+, 1 meq of K^+ and 2 meq of HCO_3. It was started at a dose of 1 mmol/kg per day in divided doses. After one week of starting therapy serum K^+ was 4 meq/l, Cl^- – 102 meq/l, pH – 7.4, and HCO_3 – 23.8 meq/l. ECG at discharge was normal. In follow-up, the patient is doing well.

Discussion

Renal tubular acidosis (RTA) is a medical condition that involves an accumulation of acid in the body due to a failure of the kidneys to appropriately acidify the urine either by failure to recover sufficient (alkaline) bicarbonate ions from the filtrate in the early portion of the nephron (proximal tubule) or by insufficient secretion of (acid) hydrogen ions into the latter portions of the nephron (distal tubule)[1].

Distal RTA (dRTA) is the classical form of RTA, characterised by a failure of acid secretion by the alpha intercalated cells of the cortical collecting duct. This leads to an inability to acidify the urine to a pH of less than 5.5. The clinical features of dRTA include[1]: normal anion gap metabolic acidosis/acidaemia, hypokalaemia, urinary stone formation (related to alkaline urine, hypercalciuria, and low urinary citrate)[2], nephrocalcinosis (deposition of calcium in the substance of the kidney) and bone demineralisation (causing rickets in children and osteomalacia in adults)[3]. The diagnosis of dRTA can be made by the observation of a urinary pH of greater than 5.5 in the face of a systemic acidaemia (usually taken to be serum bicarbonate of 20 mmol/l or less). The test usually performed is *the short ammonium chloride test*[4], in which ammonium chloride capsules are used as the acid load. Secondary causes include: Autoimmune disease (e.g., Sjögrens syndrome)[5], mutations of Band 3[6], subunits of the apical proton pump vH^+-ATPase[8], renal transplantation, sickle cell anaemia, toxins – including ifosfamide[10], toluene[11], lithium carbonate[12] and amphotericin B[13]; and chronic active hepatitis[14].

On the other hand, periodic paralysis due to hypokalaemia is often due to hypokalaemic periodic paralysis, an inherited channelopathy[15]. However, because the clinical manifestations of hypokalaemia are mainly muscle weakness, it may be difficult, in some cases, to discriminate between a paralytic attack of hypokalaemic periodic paralysis and an episode of weakness associated with hypokalaemia of another cause (e.g., reduced potassium intake, enhanced renal excretion, or digestive loss) requiring varied investigations.

In our case, all possible causes were excluded by appropriate investigations and ultimate diagnosis of d-RTA was established by ABG, 24-hour urinary potassium excretion, TTKG, NH_4Cl challenge test. Treatment with Sohl's solution was followed by rapid recovery and

patient is asymptomatic since then.

Conclusion

We report this case to focus on the diverse presentations of dRTA. The purpose of this case report is to emphasise that any patient presenting with periodic paralysis from hypokalaemia should not have a diagnostic bias and causes like thyrotoxicosis, , hyperaldosteronism, GI loss, barium poisoning, RTA, etc., should be ruled-out.

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CASE REPORT

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Successful management of rhino-cerebral mucormycosis in a patient with diabetic ketoacidosis

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Abstract

Here we report rhino-cerebral mucormycosis in a diabetic patient who was treated successfully. A 32-year-old female was brought to our hospital with altered sensorium for 1 day and fever with loin pain for 2 weeks. She had discharge from the nose and the hard palate. Microscopy of nasal scrapings showed broad aseptate hyphae and rhizopus was grown in culture. She was treated with Amphotericin B and surgical debridement was done. She recovered and was discharged. Mucormycosis should be kept in mind when treating known diabetics, as timely intervention can save the life of these patients.

Key words: Zygomycosis, immunocompromised patients, diabetes mellitus.

Introduction

Zygomycosis refers to an acute or chronic infection caused by members of the order Mucorales and entomophthorales. Mucorales cause more severe forms of zygomycosis than entomophthorales. Mucorales of medical importance include the fungi Rhizopus, Mucor, Rhizomucor, Absidia, Apophysomyces, and Cunninghamella. Mucoraceous zygomycetes are cosmopolitan in nature and are responsible for a wide range of infections which are usually infrequent but fatal[1]. Various forms of zygomycosis include rhinocerebral, pulmonary, cutaneous, gastrointestinal, renal and disseminated forms[2]. Here we report a case of rhinocerebral mucormycosis in a patient with uncontrolled diabetes mellitus.

Case report

A 32-year-old female was brought to the casualty with a history of fever for 2 weeks and altered sensorium for 1 day. The fever was associated with severe abdominal and loin pain. The patient was drowsy, not responding to verbal commands, and had minimal responses only to deep painful stimuli. There was no history of burning micturition, nausea, vomiting, cough with expectoration, or blurring of vision. Her blood pressure was 110/70 mm/Hg, respiratory rate 36/min, and pulse rate 110/min.

The patient had tenderness over the right hypochondrium. A ballotable mass was palpable in the right hypochondrium and the right lumbar area along with hepatosplenomegaly. There was no neck stiffness.

She was not a known diabetic earlier. But initial routine evaluation showed a blood sugar level of 320 mg/dl. ABG showed severe metabolic acidosis and urine showed plenty of pus cells with ketone bodies.

Ultrasonography of the abdomen showed bilateral bulky kidneys. *Escherichia coli* was grown from the patient's urine

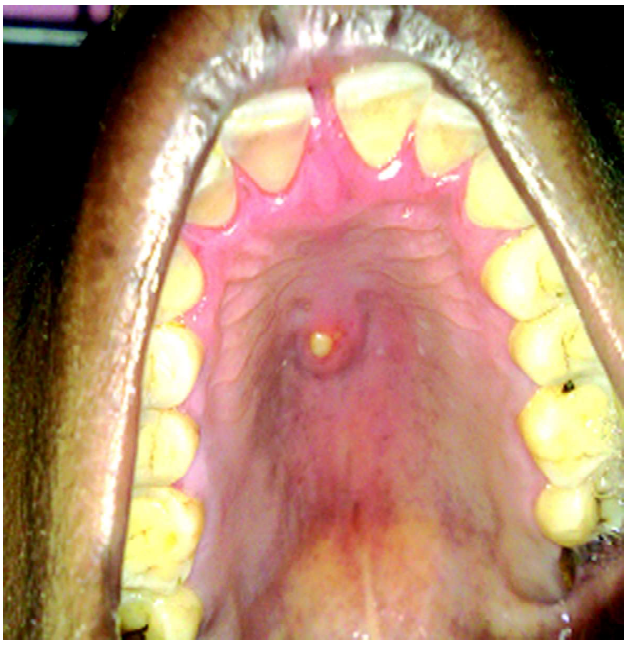

Fig. 1: *Pustular lesion on the hard palate.*

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sample which was an extended spectrum beta-lactamase (ESBL) producer susceptible to amikacin and imipenem. Blood sent for culture also grew *Escherichia coli* (ESBL) which had the same susceptibility pattern. She was managed as a case of diabetic ketoacidosis with urosepsis and was treated with imipenem and IV insulin infusion. With this the patient responded well to therapy and became conscious.

After her stay in the ICU for three days, the patient started complaining of pain in the buccal mucosa and examination showed a pustular lesion on the hard palate (Fig. 1). Scrapings from the lesion sent to the clinical microbiology laboratory showed broad, branching aseptate hyphae (Fig. 2). Endoscopic-guided scrapings from the nasal mucosa also showed the same findings. Culture of scrapings from the nasal mucosa as well as the lesion on the hard palate showed cotton woolly colonies on Sabourauds' dextrose agar within 48 hours. Lacto-phenol cotton blue mount from the colonies showed thick, aseptate hyphae with unbranched sporangiophore and oval collumella with nodal rhizoids directly beneath the sporangiophore (Fig. 3). This was identified as *Rhizopus arrhizus.*

She was treated with liposomal amphotericin B for 21 days. Endoscopy guided surgical debridement of the nasal mucosa and the hard palate lesion was done.

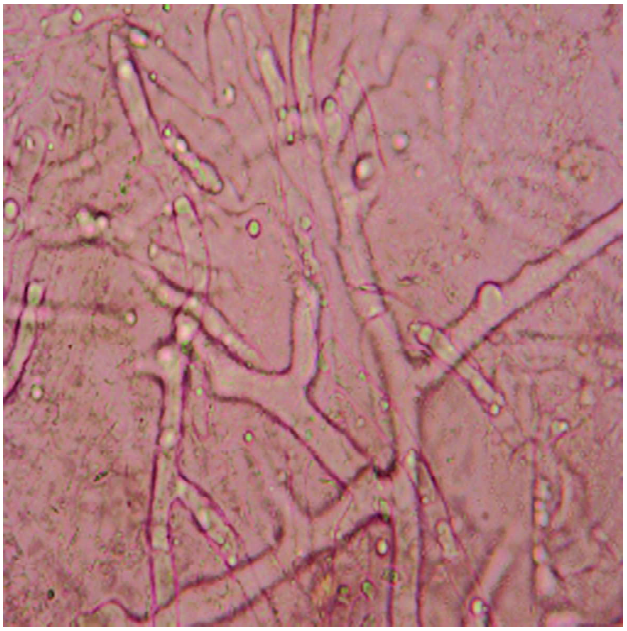

Fig. 2: *KOH mount of Nasal scrapings with broad aseptate hyphae.*

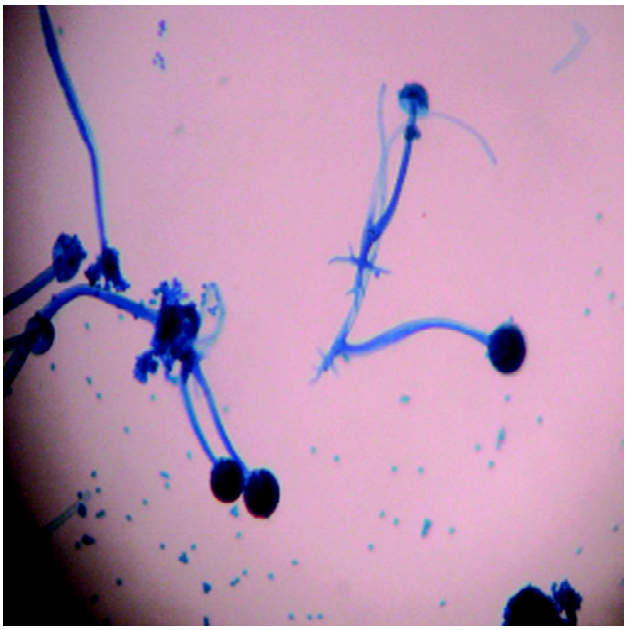

Fig. 3: *Lactophenol cotton blue mount (x 40).*

Repeat culture done from nasal scrapings after 2 weeks showed no growth. She recovered completely and was discharged after control of blood sugar levels with insulin.

Discussion

Mucormycosis has been reported from various parts of the world which includes both developed and developing countries. This is an acutely fatal fungal infection in humans and has a fatality rate of 50 – 100%[3]. Rhino-cerebral mucormycosis is the most common form of the disease. About 70 % of rhino-cerebral cases are diabetic patients with ketoacidosis[4]. Ever since the first reports of three fatal cases of advanced rhino-cerebral zygomycosis in patients with diabetic ketoacidosis in 1943 by Gregory and colleagues there are increasing numbers of these cases being reported worldwide, a majority of which are fatal[2].

Isolated renal mucormycosis has been reported from New Haven, USA[5]. In India, a case of rhino-maxillary mucormycosis has been reported from Haryana and another report of 4 cases of rhino-ocular cerebral mucormycosis has been reported from Mumbai and all these cases have occurred in diabetics[3,6]. In another study done in Chandigarh which looked at a ten-year prevalence of zygomycosis, it was found that rhino-orbito-cerebral type was the most common, followed by cutaneous and

renal among the other types. 50% of these cases were from patients with uncontrolled diabetes mellitus[7]. In Pondicherry, the cutaneous form of zygomycosis has been reported in a patient with diabetic ketoacidosis.

The spores of zygomycetes are very widely distributed in nature and they tend to grow on leaf litter and decaying substrates. Infection can occur by inhalation, percutaneous inoculation, or ingestion. These spores can cause infections in a host whose immune system is weakened by malignancy, leukaemia, diabetes mellitus, or immunosuppressive therapy[2].

Macrophages play a very important role in preventing infection in normal hosts by phagocytosis and oxidative killing of spores. But in people with uncontrolled diabetes mellitus, these cells are dysfunctional and fail to suppress the spores as they have decreased phagocytic activity due to impaired glutathione pathway[2]. An increased incidence seen in diabetics is because rhizopus are notorious in having an active ketone reductase system which thrive in high glucose and acidotic conditions. Another reason may be because of release of iron into the serum from binding proteins in ketoacidosis. The fungal hyphae produce a substance called rhizoferrin (siderophores) which when liberated into the serum binds iron avidly at low pH and the iron-rhizoferrin complex facilitates their growth by promoting major intracellular processes[4].

There are several virulence factors attributed to rhizopus which include angioinvasive nature, growth at or above body temperature, production of destructive enzymes, dormant spores being resistant to destruction at extremes of temperature, active ketone reductase system and hydroxamate siderophores[2].

Rhino-cerebral mucormycosis is the most fulminant type of zygomycosis which can be fatal even within a week if not treated. Mortality rates are very high and it was seen in a study done in Chandigarh that survival rates decline as the time interval from onset of symptoms to the time of diagnosis increases[5]. The fungi spread from the nasal mucosa to turbinate bones, paranasal sinuses, orbit, and palate, and extend into the brain where major vessels can be invaded with occlusion. These organisms have an affinity for lamina of small and large arteries causing thrombosis, haemorrhage and infarction[2].

Antifungal therapy alone or surgery alone is ineffective in treatment of rhino-cerebral mucormycosis. Treatment should involve management and correction of underlying immunocompromised state, administering appropriate systemic anti-fungal and surgical debridement. Amphotericin B given intravenously in a dosage of 0.25 - 1 mg/kg/day over 30 - 60 minutes is usually found to be effective. Toxicity to human cells is a major problem with this drug. Other modifications of amphotericin preparations that have been tried include amphotericin B lipid complex in the phospholipids form (ABLC), a lipid preparation of amphotericin B within unilamellar liposomes and a colloidal dispersion of amphotericin B. These modifications deliver higher concentrations of the drug and are less nephrotoxic[8]. Prophylactic posaconazole has been tried during an outbreak of mucormycosis with success. Granulocyte colony-stimulating factor can be administered to improve host defences and also to enhance leukocytosis[8].

Keeping in mind the gravity of the situation and the rapidity of progression of zygomycotic infections in diabetics, zygomycoses should be kept in mind when treating these patients. The key to treatment should start with treating the underlying condition coupled with surgical management and agressive antifungal therapy.

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CASE REPORT

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Acute pancreatitis as an initial manifestation of hereditary spherocytosis

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Abstract

Although gallstone disease is known to occur in patients with hereditary spherocytosis, pancreatitis has been reported infrequently in these patients. We are presenting the first case report of acute pancreatitis as the presenting feature of previously undiagnosed hereditary spherocytosis in a 45-year-old lady, who presented to our hospital emergency with severe acute upper abdominal pain. Investigations revealed haemolytic anaemia and gall stones. Elective cholecystectomy at a later date revealed multiple pigmented stones.

Key words: Haemolytic anaemia, gall stones, hereditary spherocytosis, pancreatitis.

Introduction

Hereditary spherocytosis (HS) or congenital haemolytic anaemia is a dominantly inherited haemolytic disorder characterised by anaemia, intermittent jaundice, splenomegaly and responsiveness to splenectomy. However, there is marked heterogeneity of the clinical features, ranging from an asymptomatic condition to a fulminant haemolytic anaemia[1]. Here, we have presented a case of acute pancreatitis in a previously undiagnosed 45-year-old patient of hereditary spherocytosis.

Case history

A 45-year-old female presented in the emergency with severe acute abdominal pain that was constant and radiating to the back. It was associated with nausea and vomiting. Physical examination revealed pallor, icterus, guarding and tenderness in epigastric region. Bowel sounds were reduced. Spleen was palpable 3 cm below the costal margin.

Laboratory analysis revealed anaemia, Hb of 7.2 gm/dl, leucocytosis of 13,000/mm^3, MCHC - 33.5, serum bilirubin - 2.3 gm/dl (direct - 0.6, indirect - 1.7), SGOT - 50, SGPT - 17, ALP - 206, serum amylase - 993. USG abdomen revealed multiple gallstones, spleen of 19 cm and hypoechoic pancreas. CECT abdomen revealed haziness of anterior margin of pancreatic body with adjacent peripancreatic fat streaking. She was diagnosed as a case of acute pancreatitis and managed conservatively. In view of anaemia, indirect hyperbilirubinaemia, splenomegaly and gallstones, possibility of haemolytic anaemia was kept. On further investigations, normocytic, normochromic RBCs with anisocytosis, polychromatophils, and spherocytes were seen on peripheral smear. Reticulocyte count was 6%. LDH was increased (332). G6PD assay and Coombs test were negative. Osmotic fragility revealed shift of curve to the right. The mean of the 50% hypotonic lysis test in our patient was 0.47 per cent saline. Hence the diagnosis of hereditary spherocytosis was made. Later elective cholecystectomy revealed multiple pigmented stones confirming hereditary spherocytosis as the cause of gallstones leading to acute pancreatitis.

Discussion

Hereditary spherocytosis is a relatively common haemolytic disorder in which the fundamental abnormality is an intrinsic defect of RBC membrane skeleton, and qualitative and quantitative abnormality of the spectrin or more often, in the proteins that attach spectrin to the membrane: ankyrin, band 3 and protein 4.2[1]. The disease was first recognised more than 100 years ago by the Belgian physicians Vanlair and Masius, who gave a remarkably accurate account of the disease that they called "la microcythemie". The majority of patients present with symptoms of anaemia, jaundice, or both. The diagnosis is suggested by the findings of spherocytes in the peripheral blood with negative Coombs test result. Reticulocytosis, elevated indirect bilirubin levels and elevated LDH are often present. The most useful screening test is the osmotic fragility test. Less frequently, the patient presents with one of the complications of gallstones – like cholecystitis or obstructive jaundice – and sometimes it

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may be the first manifestation of disease. Pigment gallstones develop in more than 50% of cases and incidence increases with age.

Pancreatitis as initial manifestation of hereditary spherocytosis in children is uncommon[2], but no such case has been reported in adults so far. Our patient remained asymptomatic for haemolytic anaemia and gallstones till the age of 45 years and then developed acute pancreatitis. In this extremely rare case, we diagnosed hereditary spherocytosis with the initial manifestation of acute pancreatitis retrogradely. The case is unusual because the patient was first diagnosed in the fifth decade and had initial manifestation of acute abdomen – acute pancreatitis. The mean of the 50% hypotonic lysis test in our patient was 0.47 per cent saline, which correlates with the mild-to-moderate clinical severity group[3].

Hence, hereditary spherocytosis may remain asymptomatic till late age and may present as acute pancreatitis. This disease should be suspected in a patient presenting with anaemia, splenomegaly, gallstones and/or pancreatitis, irrespective of age.

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CASE REPORT

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Type 1 aortic dissection with mesenteric ischaemia in Marfan syndrome

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Abstract

Marfan syndrome is an autosomal dominant, inherited disorder of connective tissue. It occurs as a result of somatic mutation of fibrillin I gene located on chromosome 15 and is manifested by various complications in the skeletal, ocular, cardiovascular and other systems with potential for aortic dissection and rupture. Here we report the case of a 24-year-old male patient with features suggestive of Marfan syndrome who presented with pain in the chest and back, later on involving the abdomen as well. Echocardiography revealed dilated aortic root with features suggestive of type 1 aortic dissection. CT angiography revealed features of type 1 aortic dissection with involvement of the superior mesenteric artery and left iliac artery. Patient was managed with medical therapy (β-blockers) but died before he could be taken-up for surgery – possibly due to expansion of the aortic dissection.

Key words: Marfan syndrome, aortic dissection, fibrillin gene, autosomal dominant.

Introduction

Marfan syndrome is an autosomal dominant disorder with a reported incidence of 1 in 10,000 to 20,000 individuals[1]. Patients with typical Marfan phenotype harbour different mutations of the fibrillin 1 (FBN-1) gene located on chromosome 15q-21.1[2,3]. Fibrillin 1 protein is an important component of both elastic and non-elastic connective tissues throughout the body[4,5]. Therefore, Marfan syndrome includes the ocular, cardiovascular, musculoskeletal, pulmonary, cutaneous, and the central nervous system manifestations. Prognosis in Marfan syndrome is mainly determined by progressive dilatation of the aortic root potentially leading to type 1 aortic dissection and rupture, these being the major causes of death[6,7]. Dilatation of aorta is found in 60 – 80% of adults with Marfan syndrome with dilatation of aortic root involving the thoracic aorta, abdominal aorta or even the carotid and intracranial arteries.

Case report

A 24-year-old male with insignificant past history presented early morning with sudden onset, severe and incapacitating pain in the lower chest and upper back which progressed to involve the lower back and abdomen in about 2 hours, and associated with profuse sweating. Later in the evening, the patient developed loose stools, initially watery, but later on bloody, 10 – 12 times in 24 hours. Pain in the chest and upper abdomen subsided on the 3rd day, but the patient continued to have pain in the lower abdomen (although of lower intensity). Blood with stools decreased in frequency over the next 3 days and subsided by the 5th day. The patient was initially admitted in the surgical emergency as a case of acute abdomen with a possible diagnosis of acute pancreatitis. Clinical examination revealed a conscious, apprehensive patient with a pulse of 86 bpm, regular, bounding, water hammer in quality, with a weak left femoral, left popliteal and left dorsalis pedis artery. Blood pressure in the right upper limb was 150/80 mmHg and 120/70 mmHg in the left lower limb. The patient had a tall, thin habitus with height of 183 cms, arm span 193 cms, A/H ratio of 1.06 (normal 1.05); upper segment of 83 cm with US/LS ratio of 0.83 (normal 0.93); a high arched palate, crowded teeth, sclerodactyly (Fig. 1), a positive wrist and thumb sign associated with flail joints. His chest examination revealed pectus excavatum (Fig. 2) with no ausculatory findings. In the CVS, both heart sounds were heard with a systolic murmur (3/6) at the apex associated with a musical character along with an early diastolic murmur (3/6) at A_1 and A_2. His abdominal and CNS examination was unremarkable. CBC, KFT, LFT

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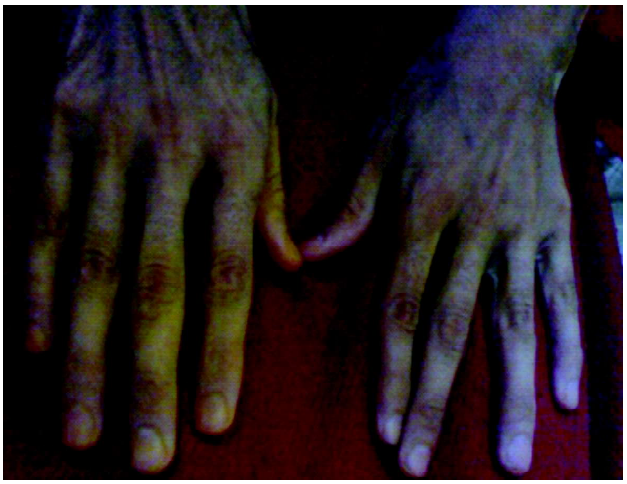

Fig. 1: *Sclerodactyly.*

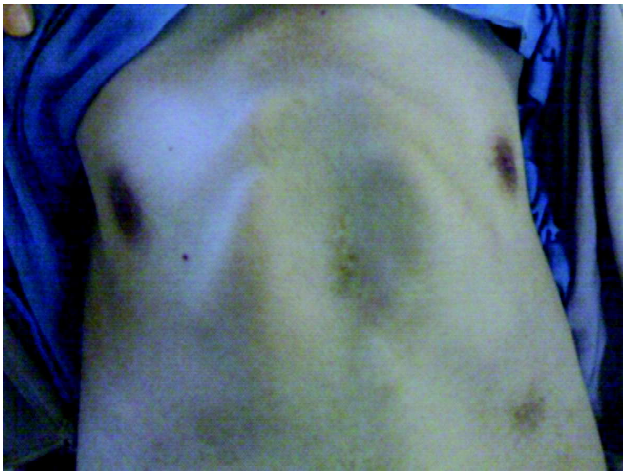

Fig. 2: *Pectus excavatum.*

and urine examination was normal. Chest X-ray revealed narrowing of ribs at places (because of chest deformity). X-ray abdomen (standing) revealed dilated small and large gut loops with thickened intestinal wall (consistent with submucosal intestinal oedema – a condition seen in ischaemic gut syndrome). USG abdomen revealed right-sided hydronephrosis with ureteric stone at the PUJ. Echocardiography performed later on (5th day) revealed dilated aortic root (44 mm) with an intimal flap seen just above the aortic valve extending down to the abdominal aorta. The mitral leaflet was thickened and redundant with moderate prolapse along with mild-to-moderate MR. LV size was normal in size and function with features of LVH. An impression of type 1 aortic dissection with severe AR, MVP, and mild MR was made on echocardiography (Fig. 3, 4, 5).

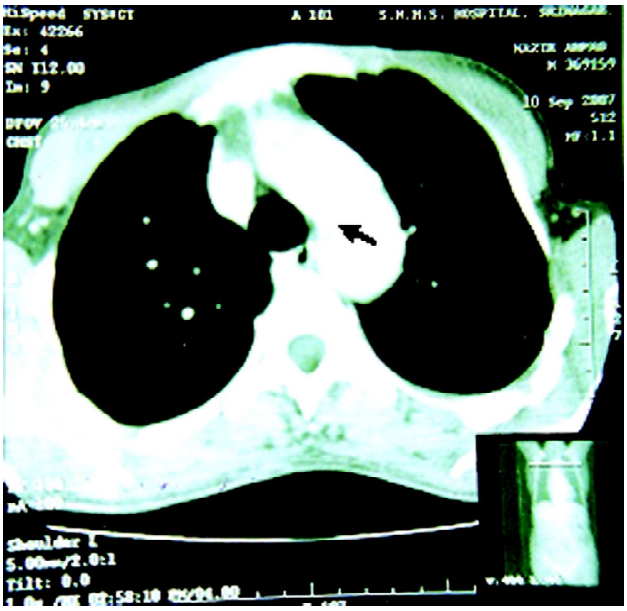

Fig. 3:

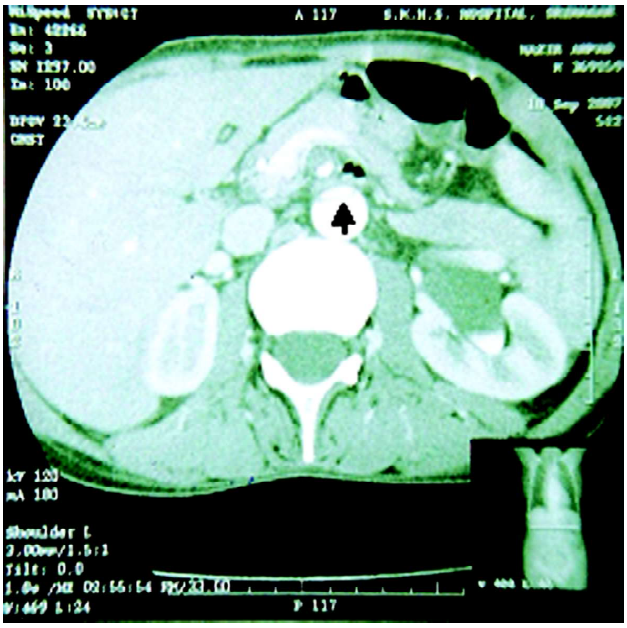

Fig. 4:

CT angiography of the chest and abdomen revealed features suggestive of type 1 aortic dissection with involvement of ascending aorta, descending aorta, superior mesenteric artery and left iliac artery along with features of bowel and mesenteric ischaemia (Fig. 6, 7, 8).

Sigmoidoscopy was deferred as the procedure involved usually leads to increase in the systolic blood pressure which was undesirable in the patient as it could have led

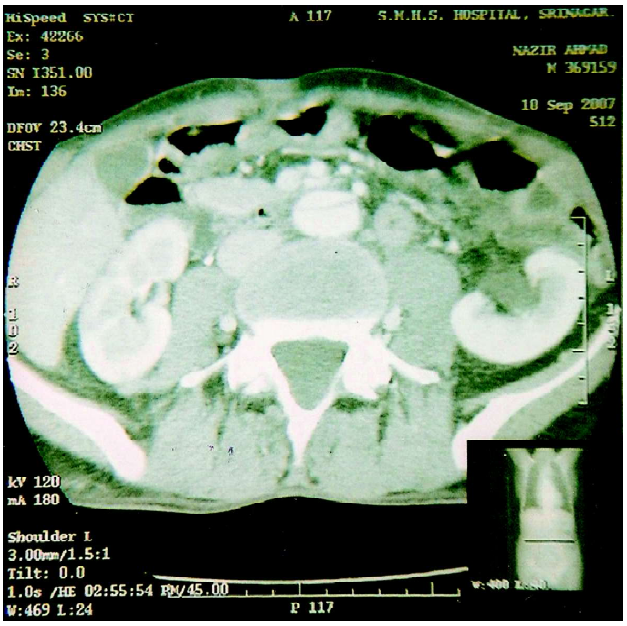

Fig. 5:

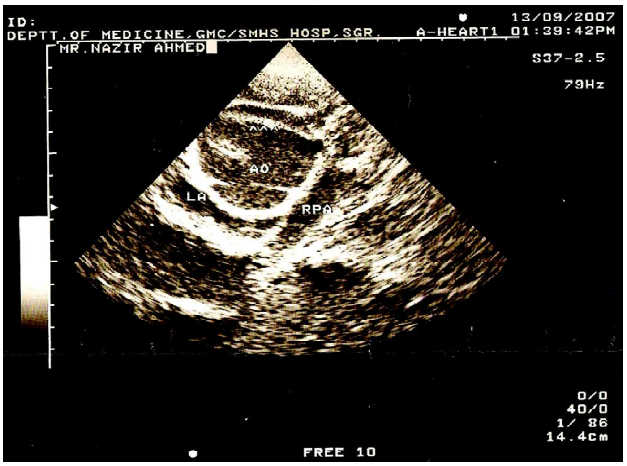

Fig. 6:

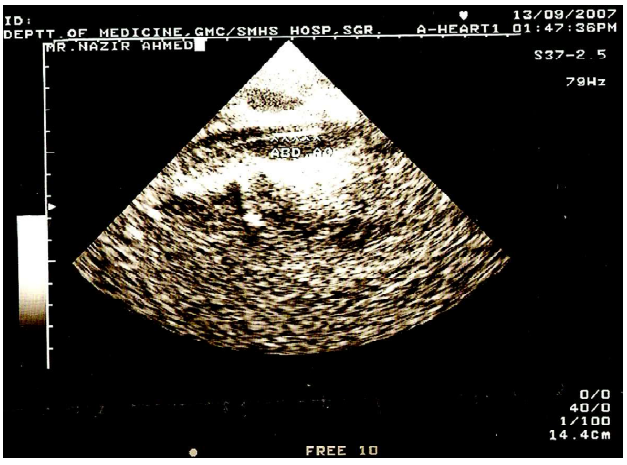

Fig. 7:

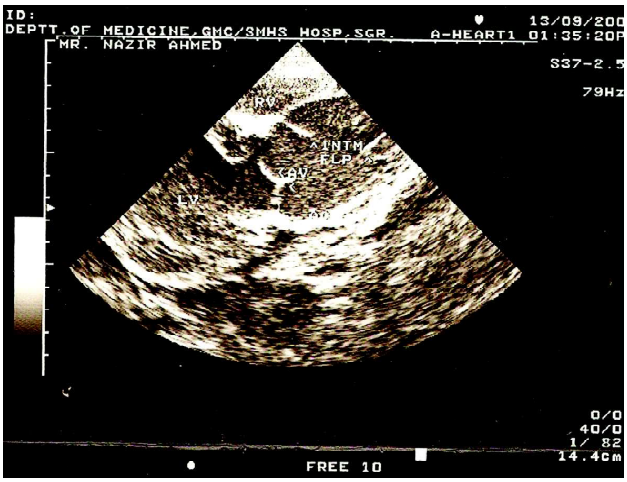

Fig. 8:

to increase in the aortic dissection. However, it was done in the third week and revealed a normal study. Slit lamp examination showed mild dislocation of the lens. Final diagnosis of the patient was Marfan syndrome presenting with AR type I aortic dissection leading to ischaemic colitis. The patient was put on nil orally, β-blockers, IV antibiotics, IV fluids, and a CVTS consultation was sought. The patient was planned for surgery. However, during the hospital stay after about 5th day patient remained haemodynamically stable with no loose motions and tolerated oral feeds without any abdominal pain. β-blockers were continued and the patient was given oral gemifloxacin also. Patient was being prepared for elective aortic reconstructive surgery; however, on the 24th day he suddenly deteriorated, developed severe abdominal pain and died possibly because of extension of the dissection.

Discussion

Marfan syndrome is clinically important because of aortic dissection which is a lethal condition affecting the aorta[8]. The risk of aortic dissection rises with increasing aortic diameter but it may also occur in non-dilated aorta[9,10]. The principal cause of early death, particularly in patients with proximal dissection is aortic rupture. The dissection could also involve branches of the aorta which obstruct the feeding vessel branch ostia giving rise to complications[8]. Mesenteric ischaemia and necrosis are potential lethal complications of distal aortic dissection. 3 – 5% of the ischaemic gut syndromes occur as a result of distal aortic dissection[11,12]. The in-hospital mortality of patients with acute aortic dissection has been reported to be 15 –

25%[13,14]. Acute mesenteric ischaemia appears to be a risk factor for early death with the overall mortality rate of > 60%[15] because operative repair for visceral ischaemia is often delayed because of difficulty in diagnosis before necrosis develops.

Life expectancy has increased significantly in patients with Marfan syndrome due to advances in medical and surgical treatment. In some patients, however, diagnosis is established only after development of aortic aneurysm, dissection or even after death[14]. All patients with Marfan syndrome are advised to take β-adrenergic blocking agents and to remain on the therapy unless intolerable side-effects develop[16]. The 30 day survival rate for patients with distal dissection treated medically is as high as 92%, the reason being that these patients often develop the extension of aortic dissection while being in hospital. Therefore medical therapy is preferred to surgical therapy for patients with uncomplicated distal dissection[8]. Surgical treatment is preferred for proximal aortic dissections and distal dissections complicated by abdominal visceral ischaemia, uncontrolled pain, and rapid expansion of the dissected aorta[8]. Patients have to be followed-up monthly for 3 to 6 months initially, 6 monthly upto 2 years, and half yearly or yearly thereafter[17].

Our case was a patient of Marfan syndrome with aortic type 1 dissection complicated by mesenteric vascular ischaemia. We could not take the patient for emergency surgery because the dissection of the aorta had got stabilised early in the course (the 5th day). Limited sigmoidoscopy using injection midazolam 3 cc IV was performed on 15th day which was normal, with monitoring of oxygen saturation and blood pressure during the procedure. However, our patient died on 24th day as a result of expansion of aortic dissection. We think the reason for such an expansion was the basic disease itself because often distal dissections are known to expand. A possibility of sigmoidoscopy being the reason for expansion of the aortic dissection was thought as the procedure would lead to increase in the blood pressure (which is an implicated cause for expansion). However, we have a compelling reason to believe that the procedure was an unlikely cause for such an expansion because the scope used was fibreoptic (much more comfortable than a rigid scope) and the procedure was done under sedation/analgesia with full monitoring of blood pressure and ABGs. The patient died 9 days after the procedure.

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CASE REPORT

JIACM 2011; 12(4) : 323-5

Histiocytic necrotising lymphadenitis disease associated with systemic lupus erythematosus

A Thukral, DN Tiwari**, K Tripathi****

Abstract

Histiocytic necrotising lymphadenitis is a self-limiting disease characterised by a painless lympadenopathy, fever, and other constitutional features. In some cases, the disease is associated with a variety of skin rashes, including cutaneous lupus, or even full blown systemic lupus erythematosus.

Key words: Histiocytic necrotising lymphadenitis (HNL), Kikuchi-Fujimoto disease, systemic lupus erythematosus (SLE).

Introduction

Histiocytic necrotising lymphadenitis was reported for the first time in 1972 in Japan by Kikuchi[1], and later on by Fujimoto and his colleagues, now popularly known as Kikuchi-Fujimoto disease (KFD). It is characterised by painless lymphadenopathy, mild leucopenia, and fever, usually affects females, and is self limiting clearing spontaneously in 1 - 4 months[1]. We are reporting a case of KFD associated with systemic lupus erythematosus (SLE).

Case report

A 24-year-old male, presented to us with fever and skin rash for two years. The fever was low grade, intermittent, and used to subside with oral antipyretics. It was associated with malaise; and except for the skin rash, there was no associated systemic complaint. The rash started along with the fever on the arms and legs; it was photosensitive and in due course of time involved the face and back. The lesions were dry, non-itching, non-scarring, papulosquamous, present on the photo-exposed areas, and also on the arms, legs and back (Fig. 1a, 1b, 1c). There were bilateral, non-tender axillary lymph nodes of size 1 - 2 cm, which the patient never noticed. Except for the rash and lymph nodes, rest of the general and systemic examination was unremarkable. There was no history of recurrent mouth ulcers, joint pains. On investigation, the patient had normal complete blood count, liver and renal function test. Urine (routine and microscopic examination) showed: albumin +, no RBC and cast, with twenty-four hour urinary protein of 700 mg/day. Anti-dsDNA and ANA titres were highly positive. Keeping in view the extensive nature of the skin rash and its association with axillary lymph nodes, both lymph node and skin biopsy was done. The skin biopsy revealed interface dermatitis, epidermal atrophy with vacuolisation of basal cells suggestive of sub-

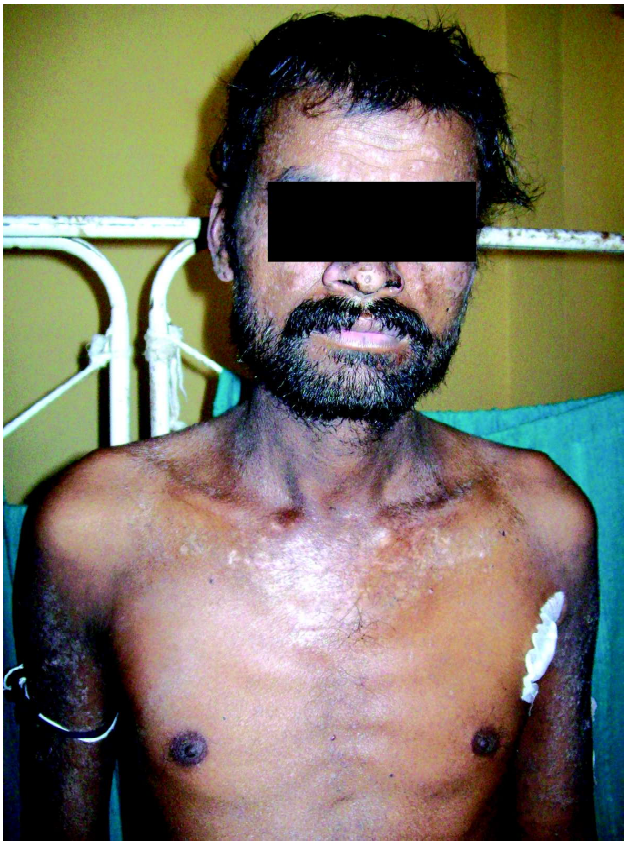

Fig. 1a: *Lesions on face and 'V' area of neck.*

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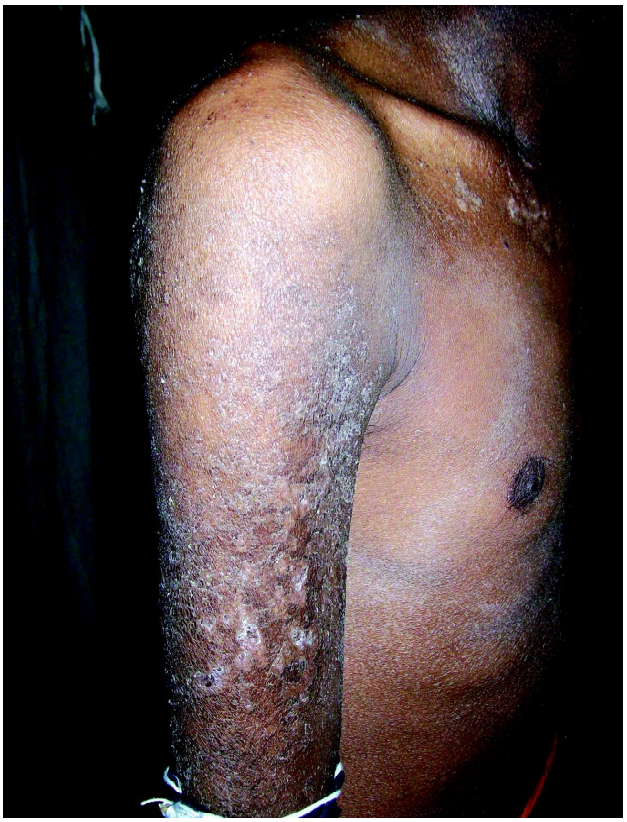


Fig. 1b: *Papulosquamous lesions on the arm.*

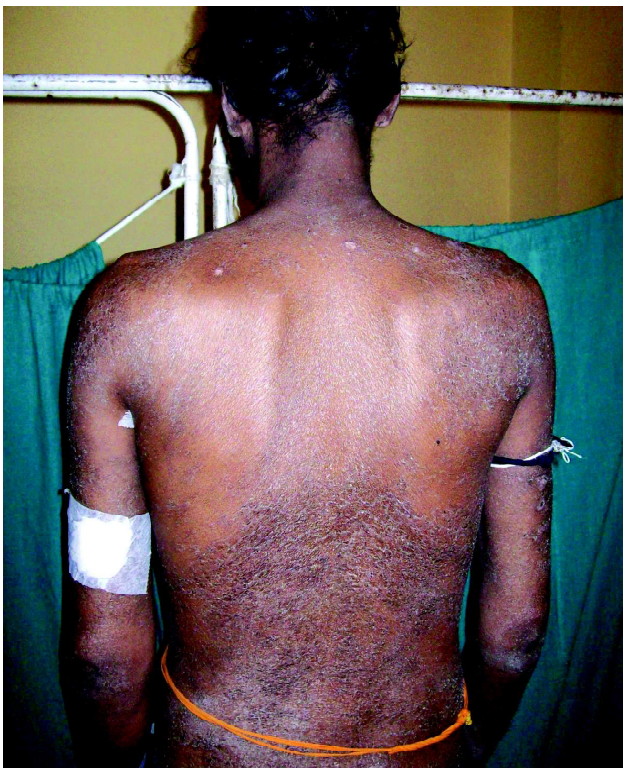


Fig. 1c: *Lesions on the back.*

acute cutaneous lupus erythematosus (SCLE)[2]. Lymph node biopsy revealed histiocytic necrotising lymphadenitis. The patient did not give consent for renal biopsy. He was managed with IV methylprednisolone, pulse cyclophosphamide, hydroxychloroquine and with medium patency topical corticosteroid and sunscreen. In follow-up, there was dramatic improvement in the skin lesions (Fig. 2a, 2b, 2c). The lymph nodes had decreased in size, and there was significant improvement in the proteinuria.

Discussion

The diagnosis of KFD is established on the basis of characteristic lymph node histopathological features, which include areas of necrosis, nuclear debris, aggregates of histiocytes, medium-to-large transformed lymphocytes and plasmacytoid T-cells, with absence of neutrophils and eosinophils[1,3], and because of these histopathological features the disease could be misdiagnosed as tuberculous lymphadenitis, leprosy, syphilis[3].

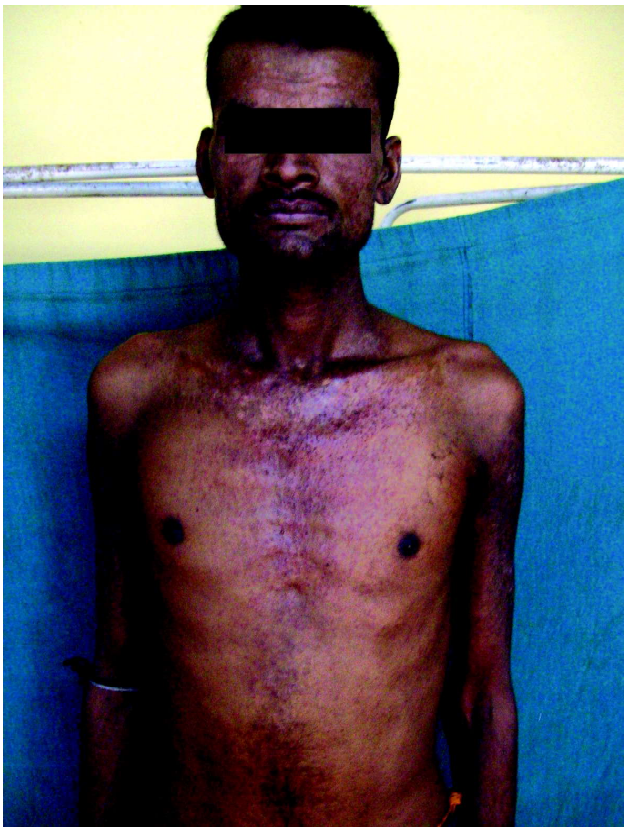
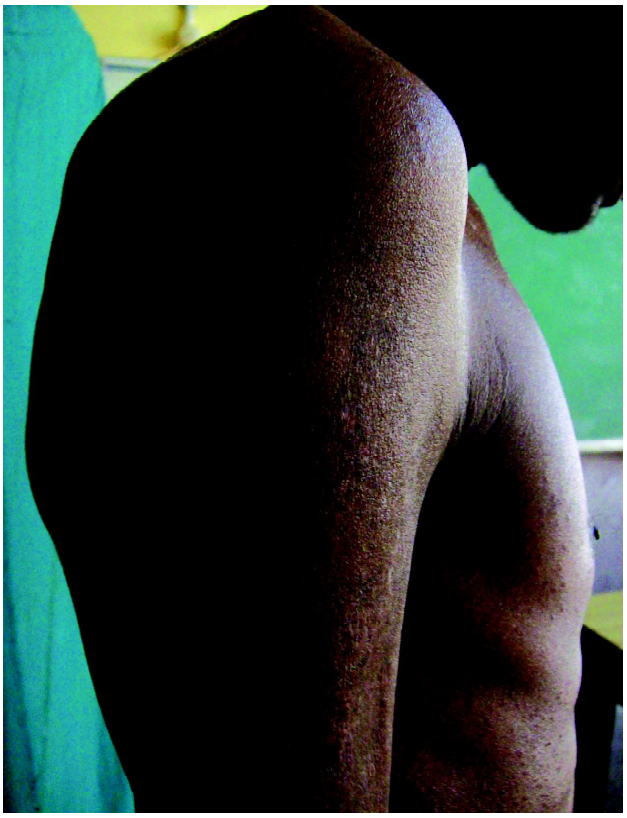
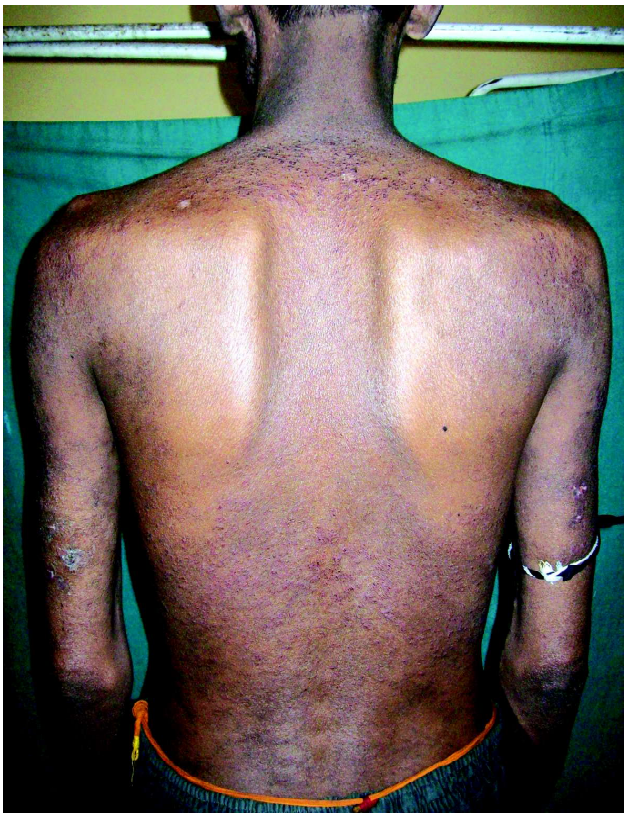


Fig. 2a: *Lesions on face and neck as seen post-treatment.*





Figs. 2b and 2c: *Lesions as seen post-treatment.*

KFD can be associated with cutaneous eruption ranging from lesions similar to drug eruption, morbilliform rash, maculopapular rash, papulosquamous or disseminated erythema[4,5,6]. Our patient had papulosquamous lesions, with histiological features suggestive of SCLE. KFD can be associated with SLE[7,8], can precede SLE[8], or can present with cutaneous lupus[9]. Our case further confirms the association of KFD and SLE, and keeping in view of the available literature, any case of KFD (a self-limiting disease) should be closely followed, as there is a possibility of manifestation of a life-threatening disease (SLE) in future.

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CASE REPORT

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Non-transmission of human immunodeficiency virus infection in persons with repeated exposure

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Abstract

There is considerable variation in susceptibility to human immunodeficiency virus (HIV) infection, with some individuals remaining sero-negative despite repeated exposure to the virus. It is estimated that three-quarters or more of all HIV infections worldwide are transmitted sexually. However, HIV transmission does not occur during every unprotected sexual contact. Infectiousness of HIV of the source partner involves several factors. We report two cases where the spouse of the HIV-infected patient remained uninfected despite repeated unprotected sexual contact over a period of up to 12 years.

Key words: HIV, sexual transmission, protection, infectivity, exposed uninfected (EU).

Introduction

HIV may be transmitted sexually when HIV in one partner's semen, cervical/vaginal fluid, or blood comes into contact with the bloodstream or mucous membranes of another partner. It is estimated that three-quarters or more of all HIV infections worldwide are transmitted sexually. However, it is clear that HIV transmission does not occur during every unprotected sexual contact. The probability of acquiring HIV-1 infection depends on the route and nature of exposure as well as the size of the viral inoculum, which relates to the viral load of the source partner. In addition, the inheritance of certain HIV-1 co-receptor polymorphisms and HLA alleles is associated with a more favourable course of HIV-1 disease and in some instances with a reduced susceptibility to infection[1]. We are reporting two patients who were HIV positive for the last 10 – 15 years and were having regular sex with their spouses. The wives of both these patients were HIV negative even after prolonged sexual exposure to HIV infection.

Case 1

A 50-year-old retired defense personnel was seen in the anti-retroviral therapy centre in our hospital in august 2007. He was diagnosed as having asymptomatic HIV infection in 1996 at army hospital. His CD4 count at the time of diagnosis was 332/mm^3. Patient was advised regarding safe sexual practices and follow-up. The patient kept his condition concealed from his spouse and other members of his family and continued having unprotected insertive vaginal intercourse with his spouse at a frequency of 2 – 3 times in a month. His latest CD4 count done in August 2007 was 198/cmm. His wife was screened for HIV by ELISA and was found to be non-reactive.

Case 2

A 48-year-old male ex-serviceman, a decorated soldier was diagnosed as HIV positive in the year 2002 at army hospital during a routine screening before leaving for a foreign mission. The patient was asymptomatic at that time. He was counselled for screening of family members, safe sexual practices, and follow-up. However, the patient did not inform his spouse or any other member of the family about his HIV status. He retired in the same year and started a shop in his native town and started staying with his family. Patient was asymptomatic for next 5 years and continued to have unprotected sexual contact with his wife in the form of vaginal insertive intercourse with a frequency of 2 – 3 times per month. He was admitted to our hospital with fever for one month in December 2007. Review of his records revealed his ELISA for HIV was reactive in February 2002. His CD4 count at the time of diagnosis was 480/mm^3 and at the time of his present admission was 254/mm^3. The patient's wife was not aware of the HIV status of the patient till the present admission. His wife underwent screening for HIV; her ELISA for HIV was non-reactive.

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Discussion

There is considerable variation in susceptibility to human immunodeficiency virus (HIV) infection, with some individuals remaining sero-negative despite repeated exposure to the virus. These exposed uninfected (EU) individuals are a useful subset of individuals with whom mechanisms that protect against HIV infection can be studied. Several host factors, either alone or in combination, might be important in conferring protection. These include host genetic factors, non-cytotoxic $CD8^+$ T-cell responses, HIV-specific cytotoxic T-cell activity neutralising antibodies to HIV co-receptors, mucosal antibodies against HIV, and increased β-chemokine production with associated $CD4^+$ T-cell resistance. In the first report from India, the T-helper and granule-mediated $CD8^+$ T-cell activities against HIV infection in a group of HIV-exposed but uninfected heterosexual partners of HIV-infected individuals were described. In that study, results indicate that HIV exposure in EU individuals might favour the generation of IL-2 and that the helper-T-cell activity observed in EU individuals not only is enhanced but also may even be greater than that in HIV-infected patients. This in turn helps maintain specific $CD8^+$ T-cells, which may be an important factor in non-transmission in EU individuals[2]. We could not investigate our patients in detail and cannot say whether any of these factors were responsible for non-transmission of infection after prolonged unprotected exposure.

Infectiousness of HIV of the source partner involves several factors. The likelihood of transmission is known to be associated with the stage of HIV disease. In the September 1994 issue of *AIDS*, Pietro Vernazza and colleagues[3] reported that people with lower CD4 cell counts and late-stage, symptomatic HIV disease were more likely to transmit the virus during sex than people with higher CD4 cell counts and asymptomatic disease. This may be related to the fact that people with later-stage disease typically have higher viral loads and more virulent strains of HIV. Both of our patients remained asymptomatic for a long time. They had a higher CD4 T-cell counts at diagnosis which persisted and were less likely to transmit the disease during the asymptomatic period. Even at presentation to the hospital this time they had a relatively higher CD4 T-cell counts (198 and 254/cmm. respectively). Researchers have also determined that susceptibility to HIV infection involves genetic co-factors. People who have two copies of a specific mutation of the gene that encodes the cell surface co-receptor CCR5 appear less likely to become infected with certain strains of HIV. People with one mutant copy of the gene tend to experience slower HIV disease progression. The frequency of the mutation differs among population groups, being higher among people of European descent and lower among people of Asian or African descent.

Phalguni Gupta of the University of Pittsburgh and colleagues[4] reported at the February 1999 CROI that semen viral load may vary widely over time in a given individual. The researchers found that in a group of 18 men, 28% did not shed HIV in their semen, 28% shed HIV consistently, and 44% shed HIV intermittently. Gupta concluded that many of the men "had very high levels of virus present in their semen at certain time points and then at other times had a lower amount... In some HIV-infected men, the levels of virus in semen can vary from one day to the next. This indicates that some men may be more likely to transmit the virus during risky sexual behaviour at certain times".

Some individuals remain inexplicably seronegative and lack evidence for human immunodeficiency virus type 1 (HIV-1) infection by conventional serologic or virologic testing despite repeated high-risk virus exposures. Zhu *et al*[5] examined 10 exposed seronegative (ES) individuals exhibiting HIV-1-specific cytotoxicity for the presence of HIV-1. They discovered HIV-1 DNA in resting $CD4^+$ T-cells (mean, 0.05 ± 0.01 copies per million cells) at multiple visits spanning 69 to 130 weeks in two ES individuals at levels that were on average 10^4- to 10^6-fold lower than those of other HIV-1-infected populations reported. Sequences of HIV-1 envelope and *gag* genes remained markedly homogeneous, indicating little to undetectable virus replication. These results provide the evidence for HIV-1 infection in ES individuals below the detection limit of standard assays, suggesting that extraordinary control of infection can occur.

In a recent study, Castro *et al*[6] found changes of both CD4+

and CD8+ T-cells in highly HIV- exposed, uninfected individuals, with a lower level of naïve and CD28+ T-cells and higher levels of HLA-DR+ T-cells and CD4+ T-cells expressing CCR5 and memory $CD4^+$ T-cells than in control subjects. These data suggest that the peripheral immune cells of highly exposed, uninfected individuals responded according to the level of HIV exposure from the partner, even though evidence of specific HIV stimulation is rarely seen.

Any of the factors mentioned above or some yet unidentified factors might have been responsible for non-transmission of infection in our patients. However, there is no evidence as yet that natural immunity to HIV-1 infection exists and can protect against infection. If such immunity could be identified, the findings would have major relevance to vaccine development.

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CASE REPORT

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Acute lead encephalopathy in an adult with dimercaprol-induced Stevens-Johnson syndrome

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Abstract

A 40-year-old male, working in a factory involving re-melting lead batteries developed acute gastrointestinal symptoms followed by seizure and encephalopathy. Clinical history of exposure, high serum lead level, similar gastrointestinal symptoms in co-workers, and response to D-penicillamine suggested the diagnosis of lead encephalopathy. However, he developed Stevens-Johnson syndrome (SJS) after introduction of dimercaprol. This complicated condition was further managed conservatively with good clinical outcome. Temporal relationship of SJS with dimercaprol suggests a cause and effect relationship, which is not a common reported complication of dimercaprol.

Key words: Lead encephalopathy, adult, D-penicillamine, dimercaprol, Stevens-Johnson syndrome.

Introduction

Acute lead encephalopathy is very rare in adults[1, 2]. Despite measures to reduce and control lead use, serious lead intoxication still occurs as an occupational hazard in unmonitored small scale industries, particularly in the developing countries. Heavy metal chelators are the treatment of choice, but these are not free from side-effects. We report a case of acute lead encephalopathy, who developed Stevens-Johnson syndrome (SJS) after chelating therapy and improved on conservative therapy.

Case report

A 40-year-old male, working in a factory involving re-melting lead batteries for the last one month, presented with a three-days history of colicky abdominal pain, constipation, anorexia, nausea and vomiting. On day four of the illness, he developed an episode of generalised seizure followed by altered sensorium. There was no history of fever, headache preceding this episode; nor was there any history of change in urinary colour. He denied history of indigenous drug exposure, addictions, substance abuse, or similar complaints in the past. Few of his co-workers also had experienced similar, severe abdominal pain, in the last few months, but none had altered behaviour, change in sensorium, or seizures.

On general physical examination, he was afebrile and had pallor, mild icterus, and conspicuous blue lines on the gums. He was conscious but disoriented and restless. Rest of the examination of his nervous system including cranial nerves and motor-sensory system was normal. There were no signs suggestive of meningeal irritation. There was no abdominal tenderness or organomegaly.

On the basis of clinical features and history of occupational exposure, a provisional diagnosis of acute lead encephalopathy was made. On investigation, it was supported by high serum lead: 130.6 microgm/dl (normal < 25 microgm/dl) and urinary lead levels: 1,343 microgm/L (normal: < 80 microgm/L). Other investigations revealed microcytic hypochromic anaemia with haemoglobin 8.5 gm/dl, total leukocyte count 18,500/mm^3 with polymorphonuclear pleocytosis and normal platelet counts. Liver function tests were deranged with serum total bilirubin 3.2 mg% (direct bilirubin 1.6 mg%), normal serum AST, ALT, and alkaline phosphatase. CSF examination revealed pleocytosis with total 80/mm^3 cells (90% polymorphonuclear cells), normal sugar and raised protein (129 mg/dl). Renal function test, blood culture, abdominal ultrasound were all normal. MRI brain did not reveal any significant abnormality. Scalp electroencephalogram revealed nonspecific diffuse slow waves.

Based on the diagnosis of acute lead encephalopathy, he was started on D-penicillamine 250 mg once daily, which was increased by 250 mg every 4th day, and within 2 days he started showing clinical improvement. Due to initial unavailability, injection Dimercaprol (4 mg/kg, 4 hourly)

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was started on day 10 of treatment to hasten recovery. However, next day he developed generalised itchy erythematous rash with oral mucosal ulceration suggestive of SJS. D-penicillamine and dimercaprol were stopped and patient was managed symptomatically. His serum and urinary lead levels, repeated two weeks after initiating treatment, were reduced (61.0 microgm% and 154.7 microgm/l respectively), and he was discharged after 4 weeks of hospital stay in an asymptomatic state and without any neurological deficit.

Discussion

Lead poisoning as an occupational hazard was first described by Hippocrates in a case of a metal extraction worker with abdominal colic. Mass screening in the US in the 1980s disclosed elevated blood lead levels in 2% of the general population, levels being higher in children than in adults. The Control of Lead at Work Regulations had prescribed safety limits for occupational lead exposure in 1998, according to which blood lead values <1.45 micromol/l (30 microgm/100 ml) represent reasonably well-controlled occupational exposure provided there is 6-monthly monitoring, 1.45 – 2.4 micromol/l (30 – 50 mg/100 ml) require investigative action by the employer and 2.4 – 2.9 micromol/l (50 – 60 mg/100 ml) call for suspension of the worker from exposure. However, US CDC and WHO recommends blood lead level > 0.5 micromol/l (10 microgm/100 ml) as cause for concern. Since the measures to reduce and control lead use were taken the incidence and prevalence of lead poisoning is much reduced.

Lead poisoning most often occurs in 1- to 3-year-old children due to chewing of lead paint. Acute encephalopathy is the commonest presentation in children, which can be fatal or can lead to permanent neurological sequelae.

Lead toxicity is much less common in adults, and it is mainly an occupational hazard due to inhalation of lead fumes or physical contact with lead in processes that require remelting of lead, such as painting, printing, lead smelting, pottery glazing, welding, and storage battery manufacture.

The usual manifestations of lead poisoning in adults are colic, anaemia, and peripheral motor neuropathy. Encephalopathy is rare in adults[1, 2]. The increased resistance is believed to be due to the increased capacity of the mature brain to sequestrate lead away from its mitochondrial site of action within the cerebral and cerebellar neurons[3].

Whitfield *et al* studied 23 patients with lead encephalopathy and reviewed 31 similar patients from 1961 – 1967[4]. All 23 patients had history of consumption of illicit liquor contaminated by lead (moonshine). Clinical features included seizures (82%), altered sensorium (44%), lateralising neurologic signs (35%), dizziness, syncope, disorientation, and blindness. Other features are anaemia (91%), basophilic stippling (70%), lead line (47%), increased urinary lead excretion (100%), mild CSF pleocytosis (5 to 27 WBCs/mm^3) with raised CSF protein (50 to 185 mg%) in 63%, and response to ethylene diamine tetraacetic acid (EDTA) in 87% patients.

The following criteria have been suggested by Whitfield *et al* for diagnosis of lead encephalopathy: 1) Documentation of diffuse encephalopathy with or without focal signs; 2) Diagnosis of lead toxicity using clinical and laboratory aids; 3) Response to chelators; and 4) Exclusion of other causes of encephalopathy[4].

In our patient, a diagnosis was made on the basis of history of occupational exposure, similar abdominal complaints in co-workers, history of abdominal colic, presence of anaemia, lead lines on gum, elevated blood and urine lead levels, and the dramatic response to chelator therapy (both clinical and biochemical) and exclusion of other causes.

The main principles of management are to remove the patient from the environment of lead, good hydration, use of chelators, mannitol, and/or dexamethasone for raised intracranial tension and anti-convulsants for seizures. Dimercaprol, edetate calcium disodium, and Succimer are the main agents primarily used for chelation; however, penicillamine has also been used with good clinical outcome[5, 6]. Our patient tolerated penicillamine well with clinical improvement. Patient developed SJS after adding dimercaprol to hasten recovery, which is not a reported adverse reaction of dimercaprol. Only one case of SJS with oral dimercaptopropane-1-sulfonate (DMPS, chemically

similar to dimercaprol) therapy has been reported but none with dimercaprol[7, 8]. Temporal association of dimercaprol with SJS in the present case suggests a cause-effect relationship.

Our patient was probably one of the rare cases of SJS with dimercaprol. Another uncommon feature was presence of jaundice, which has not been mentioned earlier with lead toxicity[4, 5].

High index of suspicion is required to make a diagnosis of lead encephalopathy, so as to institute early timely treatment. Education of the public regarding the hazards of lead exposure and follow-up of the high-risk individuals is mandatory for prevention. Thus we report an uncommon case of lead encephalopathy in an adult who had a good clinical response to oral therapy and also rare association of SJS with dimercaprol.

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CASE REPORT

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Isoniazid-induced gynaecomastia

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Abstract

Gynaecomastia as a side-effect of some drugs is quite common; but due to anti-tuberculous drugs it is very rare. We report a case of a male patient who was diagnosed as a case of Koch's lung and was started on anti-tuberculous drugs. While taking anti-tuberculous drugs he developed gynaecomastia and after withholding isoniazid, his breast enlargement subsided.

Key words: Gynaecomastia, isoniazid, drug-induced gynaecomastia.

Introduction

Side-effects of isoniazid are fairly common, e.g., hepatotoxicity and peripheral neuropathy[1]. Isoniazid is a low-cost and effective anti-tuberculous drug. Other side-effects of isoniazid are nausea, vomiting, dry skin, fever, acne, pruritus, leukopenia, thrombocytopenia, anaemia, lymphadenopathy, high blood sugar, ringing in the ears, urinary retention, painful joints, psychosis, seizures, encephalopathy, optic neuritis, optic atrophy, and impaired memory[2].

Although isoniazid is implicated as a cause of gynaecomastia, the literature on this is rare. Online search showed only a few case reports from India[3], two reports from France[4,5], and one from Italy[6]. The first report from France was published in 1953 and the second report on it was from Italy in 1957. Here we are reporting a case of isoniazid-induced gynaecomastia in which isoniazid was used in a therapeutic dose (300 mg).

Case report

A 28-year-old male, farmer by occupation, presented to our outdoor department. He was a non-smoker, non-alcoholic, and had complaints of mild fever more so in the evenings, without chills and rigors, with cough which was white in colour. His appetite was decreased for last 2 - 3 months. He also complained of two episodes of haemoptysis one month back. On examination, our patient had crepitations in the right supra-mammary, upper axillary, and supra-scapular areas. On investigation, the patient's haemoglobin was 09 gm/dl; total leukocyte count: 10,400; differential count: N-80, L-19, M-1; urine routine and microscopy, LFT, and RFT were normal. Chest X-ray showed Koch's lesion in right upper lobe. On further evaluation he was diagnosed as a case of sputum-positive pulmonary tuberculosis. For this, the patient was prescribed anti-tuberculous drugs (under category I) with isoniazid (300 mg), rifampicin (450 mg), pyrazinamide (1,500 mg), ethambutol (800 mg) for two months, followed by HRE for 4 months. After 3 months of initiation of the treatment, patient did gain weight of 4 kg (from 46 kg to 50 kg), but he developed painful enlargement of both breasts. He had bilateral tender mobile breast lumps 3 x 4 cm in diameter. His mammogram showed bilateral benign mammary tissue hyperplasia. On examination, his secondary sexual characters and external genitalia were found to be normal. His hormone levels: TSH: 2.4 IU/ml (0.27 - 4.2 IU/ml), LH: 3.8 mIU/ml (1.7 - 8.6 mIU/ml), FSH: 4.2 mIU/ml (1.5 - 12.4 mIU/ml), prolactin: 7.8 ng/ml (1.6 - 18.7 ng/ml), oestradiol: 65 pg/ml (< 20 - 77 pg/ml) and testosterone: 5.4 ng/ml (2.8 -8.0 ng/ml) were normal. There was no change in libido and sexual function. On suspecting the cause of gynaecomastia to be drug-induced, we withheld isoniazid but continued the rest of the anti-tuberculous drugs. After 2 months of stopping the isoniazid, the swelling and pain subsided in both breasts.

Discussion

Gynaecomastia is the enlargement of the male breast[7]. It was first described by Pauls Aegineta (AD 625 - 690) who thought it to be due to the formation of fat[8]. Gynaecomastia is caused by excess oestrogen action

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which is usually a result of increased oestrogen/androgen ratio[7].

Gynaecomastia occurs as a normal physiological phenomenon as in newborns (due to transplacental transfer of maternal and placental oestrogen), puberty (high oestrogen and androgen ratio in early stages of puberty) or ageing (increased fat tissue and increased aromatase activity), or as some pathology[7].

Pathological causes can be a drug-induced; increased conversion of androgen to oestrogen as in congenital adrenal hyperplasia, hyperthyroidism, liver cirrhosis; excess oestrogen as in sertoli cell tumour; or androgen deficiency as in Klinefelter syndrome, androgen insensitivity syndrome.

Drugs are a very important cause of gynaecomastia[9,10]. Few of them are diethylstilbesterol, digitalis, clomiphene, phenytoin, spironolactone, ketoconazole, metronidazole, cisplatin, finasteride, diazepam, methyldopa, phenothiazines, cimetidine, captopril, metoclopramide, methotrexate, etc. Most of these drug-induced gynaecomastias were reported through various case reports[11]. Here we have reported a case of gynaecomastia-induced by isoniazid.

Gynaecomastia is a very rare side-effect of isoniazid. It has been hypothesised that disturbance in vitamin B6 complex activation in liver leads to altered oestrogen and androgen metabolism. The physiologically active form of vitamin B6, pyridoxal 5-phosphate (PLP), is known to function as a co-factor in many enzymic reactions in amino acid metabolism. Apart from its role as a co-enzyme, PLP acts as a modulator of steroid hormone receptor-mediated gene expression. Specifically, elevation of intracellular PLP leads to a decreased transcriptional response to glucocorticoid hormones, progesterone, androgens, and oestrogens[12].

It has also been postulated that isoniazid probably acts by a phenomenon called "Refeeding Gynaecomastia", which is supposed to be caused by restoration of weight, gonadotrophin secretion, and gonadal function[13]. Among the anti-tuberculous drugs, isoniazid, thioacetazone and ethionamide have been implicated as a cause of gynaecomastia[14,15].

Most patients with gynaecomastia generally require no treatment other than the removal of its cause. In case of drug-induced gynaecomastia, discontinuing the drug is the best cure. Specific treatment of gynaecomastia is needed when it is causing embarrassment, pain, or emotional discomfort and thus interfering in the patient's daily life. In such cases, surgery can be considered. In cases where surgery is not possible, anti-oestrogens (tamoxifen) or aromatase inhibitors (testolactone) can be used[7].

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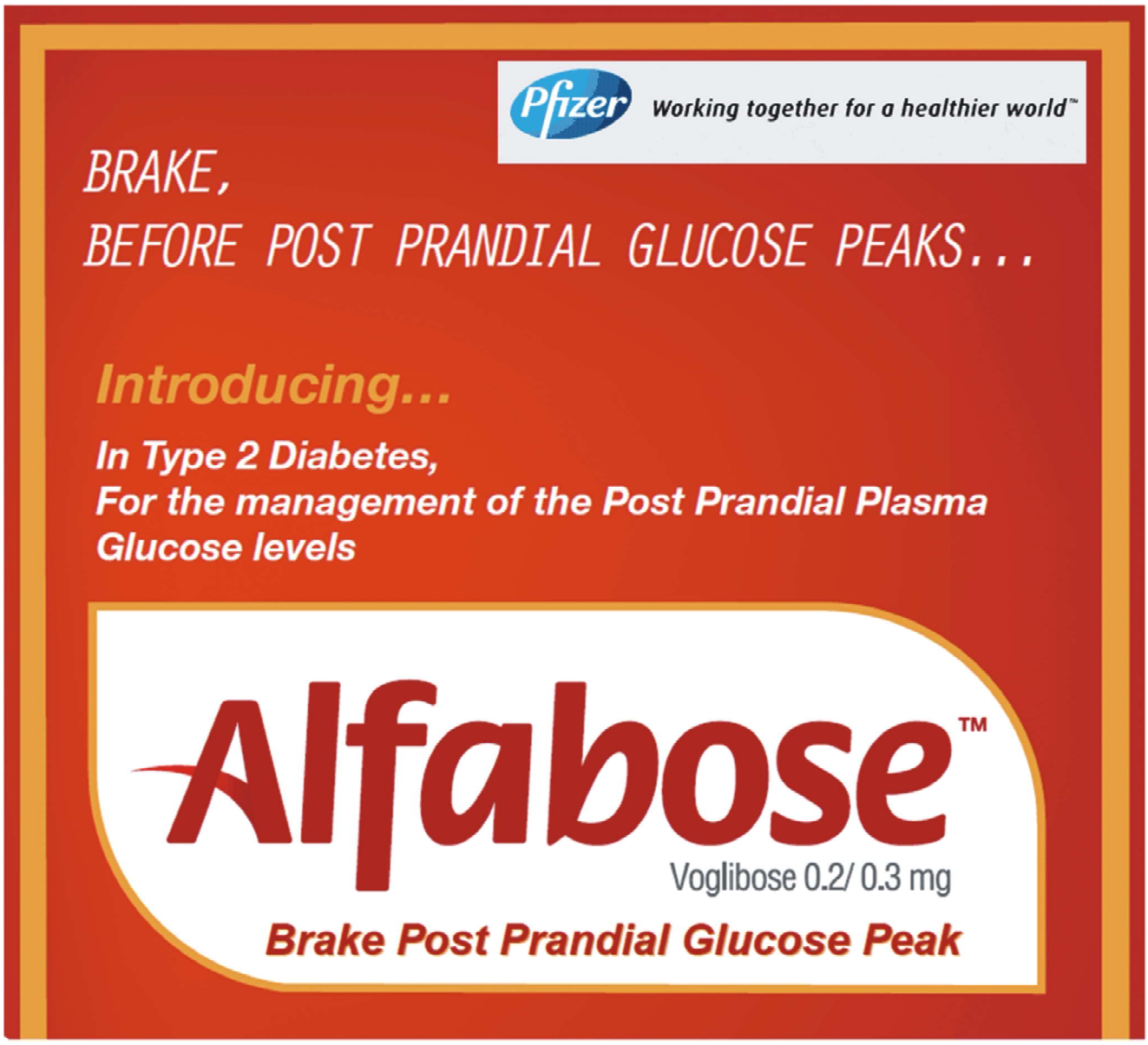
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