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HEMATO ONCOLOGY

NUTRITIONAL ANEMIA

Thilagavathi V

Abstract: *Nutritional anemia in children is a common deficiency disorder and iron deficiency is the most common cause manifesting as either isolated or combined deficiency. Iron plays an essential role in hemoglobin synthesis and B 12 and folate in DNA synthesis. Inadequate intake of foods rich in iron, B12 and folate, malabsorption, infections and inflammation cause the state of deficiency. It is important to identify the specific cause of anemia and treat appropriately.*

Keywords: *Anemia, Nutritional, Iron, Folate, B12, Child.*

Nutritional anemia continues to be a common deficiency disorder, especially in developing countries and occurs due to deficiency of one or more of the essential nutrients required for the synthesis of hemoglobin namely iron, vitamin B12, folic acid, vitamin C, vitamin E and proteins.[1] Iron is the most important component of hemoglobin. Iron deficiency is the most common cause of nutritional anemias and commonly occurs during periods of increased requirement, e.g. in infancy, adolescence, pregnancy and during lactation especially among people with poor socioeconomic status due to inadequate intake of dietary iron, infestations, infections and malabsorption. Nutritional anemia due to folic acid deficiency is more common than vitamin B12 deficiency.[1] The incidence of iron deficiency anemia in preschool children and up to the age of 14 years is estimated to be 48%. The prevalence of vitamin B12 deficiency is important but not a commonly recognized cause of nutritional anemia.[2]

Erythropoiesis is the process in which new erythrocytes are produced. Erythroblasts require large amounts of iron for the synthesis of hemoglobin, vitamin B12 and folic acid for proliferation during their differentiation. Deficiency of vitamin B12 and folic acid inhibits purine and thymidylate synthesis, impairs DNA synthesis and erythroblast apoptosis resulting in nutritional anemia of ineffective erythropoiesis.[3] Many proteins are found to be involved in absorption, cellular export of non-heme iron and in erythroblast uptake and utilization of iron.

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Iron deficiency anemia

Iron requirement

The total body iron stores at birth in a term newborn and the iron obtained from the fall of hemoglobin during the first 3 months of life is sufficient for the blood formation during the first 6 to 9 months of life. The iron requirements in children of various age groups are listed in the table below.

Etiology

Inadequate dietary intake of iron is the major cause of iron deficiency anemia, commonly occuring at 9 to 24 months of age and is relatively uncommon thereafter.[1,3] Nutritional iron deficiency anemia is common in overweight children who consume excessive cow's milk. Inadequate dietary intake and faulty food habits like excessive intake of milk and milk products are the most common cause of iron deficiency anemia. Iron deficiency is common in undernutrition, blood loss from G.I tract (peptic ulcer, Meckels' diverticulum, polyp, hemangioma or inflammatory bowel disease, cow's milk protein allergy). Infections with hookworm, Plasmodium, Trichuris trichiura, Giardia lamblia and Helicobacter pylori cause iron deficiency anemia.

Pathophysiology

Iron plays an essential role in the synthesis of hemoglobin. It is unique in that it takes up and releases oxygen with no energy expenditure. Large amounts of iron are recycled daily from the breakdown of hemoglobin from the destroyed red cells. Dietary iron occurs in two forms. Heme iron from animal proteins is better absorbed than non-heme iron. Non-heme iron is obtained from plant foods and vegetables and is absorbed in the ferrous form mostly in the duodenum and to a lesser extent in the jejunum and proximal ileum. Absorbed iron from the intestinal lumen is transported with a divalent metal protein (DMT 1) across

Table I. Iron requirement in children of various age groups [4, 5]

Age	Amount of iron per day (recommended daily allowance)
0 to 6 mo (term)	0.27mg
0 to 12 mo (preterm)	2-4mg/kg
7 to 12 mo	11 mg
1 to 3 years	7 mg
4 to 8 years	10 mg
9 to 13 years	8 mg
14 to 18 years	11 mg (for boys) 15 mg (for girls)

the mucosal border. From the enterocyte the iron reaches the plasma by the specific transporter protein called ferroportin. The primary mechanism of iron homeostasis is regulated by hepcidin.

Hepcidin level decreases in iron deficiency and increases in inflammation and iron excess. The elevated hepcidin blocks the ferroportin and limits the mobilization of iron in to the plasma. Iron circulates in the blood bound to transferrin. Bone marrow erythroblasts have receptors for the iron transferrin complex and the iron complex enters the cells by endocytosis for hemoglobin synthesis. The absorption of iron is regulated by iron deficiency (absorption increases), inflammation and iron repletion (absorption decreases), mediated by hepcidin.

Over 90% of the dietary iron for infants and young children are non-heme iron. Only 10% of dietary non haem iron is absorbed. The absorption of non- heme iron is highly altered by the dietary factors. Foods rich in Vitamin C like orange juice, meat and fish enhance iron absorption; calcium, phosphates, tannin in tea and bran decrease the absorption. Breast milk iron, present in low concentrations is well absorbed; cow's milk contains high levels of calcium and phosphorus that interfere with iron absorption.

Clinical manifestations

Nutritional iron deficiency anemia develops gradually and children remain mostly asymptomatic. Pallor develops when the hemoglobin falls below 8gm/dL. In severe iron deficiency anemia (hemoglobin below 5g / dL) and children present with irritability, anorexia and lethargy. High output cardiac failure occurs in severe anemia. Iron deficiency anemia in the first 2 years of age is associated with developmental delay and poor cognitive function. It has been proved by several studies that cognitive defects due to iron deficiency are irreversible. Severity of the neuro cognitive defects is directly proportional to the severity of the anemia.[6] Pica is a common symptom which predisposes to consumption of lead resulting in plumbism. The long term consequences of iron deficiency are poor growth and development, depressed immune function and behavioural changes.[7]

Laboratory diagnosis

Complete blood count (CBC) reveals decreased mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). The red blood cells are microcytic and hypochromic with anisopoikilocytosis. The reticulocyte count is normal. The body iron status is assessed by the serum iron, iron binding capacity, transferrin saturation and ferritin. It is known that iron level is susceptible to diurnal variation hence it is not recommended for the diagnosis of iron deficiency anemia. Red cell protoporphyrin is elevated in iron deficiency but is less specific. Bone marrow aspiration for iron stores is the best indicator of iron status but it is an invasive procedure. Serum ferritin reflects the iron status but is elevated in infections and inflammatory conditions. The committee on nutrition of the American Academy of Pediatrics recommends the hemoglobin <11gm/dL and serum ferritin of <10 mcg/L as diagnostic of iron deficiency anemia in the presence of normal CRP. The reticulocyte hemoglobin content (CHr) is decreased (27.5) and serum transferrin receptor 1(TfR1) is increased in iron deficiency.[8] These two parameters are not affected by infection or inflammation and hence considered as ideal indicators of iron deficiency.

The other causes of microcytic anemia are thalassemia and anemia of chronic disease. Thalassemia is differentiated by normal red cell distribution width, elevated serum

ferritin and the increased fetal hemoglobin. Anemia of chronic disease is usually normocytic can be microcytic at times.

Treatment

Oral iron therapy is the corner stone of management. Ferrous sulphate is preferable as it has maximum relative bio availability and is soluble in water. Ferrous gluconate has equal bioavailablity and is also recommended for treatment.[8] The recommended daily dose of elemental iron is 3 to 6mg/kg/day in 2 to 3 divided doses for a minimum period of 3 months. The drug taken on an empty stomach or 1 to 2 hrs before or after meals to maximize the absorption. The absorption of iron can be enhanced by administering the drug along with fruit juice or vitamin C.

The response to iron therapy is monitored by haemoglobin levels. An increase of 1gm/dL after 1 month of oral iron is the most practical means to establish the diagnosis. After normalisation of haemoglobin it requires a minimum of 3 months for the repletion of iron stores. The most common cause of treatment failure is poor compliance. Parental iron is indicated only in malabsorption. Iron sucrose, ferric carboxy maltose and ferric gluconate are the preferred parenteral preparations as they have a lower risk of serious reactions.[9] It is reported that iron therapy for a longer period may be tried in cognitive defects associated with iron deficiency.

Folic acid deficiency anemia

Folic acid or pteroylglutamic acid is not biologically active but the reduced form tetrahydrofolates are biologically active. Folates are not synthesised by the body and depend on the dietary sources for the requirement. These biological compounds are heat labile and water soluble, present in fruits, green leafy vegetables, liver and kidney. Dietary forms of folic acid are in the form of polyglutamates which are hydrolysed to pteroylmonoglutamic acid in the mucosal cells of the small intestine. Folic acid and vitamin B12 play an important role in purine and pyramidine biosynthesis. Folate deficiency results in reduced purine and pyramidine biosynthesis and impaired DNA synthesis and cell division leading to ineffective erythropoisis.

Causes of folate deficiency

Daily requirement of folate is 100 to 200 mcg. Anemia due to folate deficiency occurs due to inadequate intake of foods rich in folate and consumption of goats milk which is deficient infolic acid. Excessive boiling and pasteurization result in loss of 75% of the folate. Folate deficiency can occur in malabsorption syndromes and protein energy malnutrition. Intake of drugs like phenytoin, methotrexate and pyrimethamine cause folate deficiency.

Clinical manifestations

Folate deficiency causes megaloblastic anemia. Body stores are sufficient for only four months and anemia due to folate deficiency occurs at 4 to 7 months of age, much earlier than iron deficiency. Preterm infants are at higher risk of developing folate deficiency due to inadequate folate stores. They usually present with irritability, poor feeding and growth retardation. Several studies have proved that folate deficiency during periconceptional period leads to neural tube defects. Combined folate and vitamin B12 deficiency in infants presents with abnormal behaviour and defective cognitive and motor function.[10]

Laboratory diagnosis

The peripheral smear shows macrocytic RBCs with aniso poikilocytosis and hypersegmented neutrophils. Neutropenia and thrombocytopenia are seen in severe deficiency. The bone marrow aspiration is hypercellular with prominent megaloblastic changes in the erythroid precursors and in the granulocytic precursors. Normal serum folic acid levels are 5-20ng/mL; and in deficiency it is <3ng/ml. The normal RBC folate is 150 to 600ng/mL of packed cells. The RBC folate is a reliable indicator of chronic deficiency and <50ng/mL is diagnostic.

Treatment

Folic acid 0.5 to 1mg/day for 3 to 4 weeks is given orally or parenterally. The initial response to folate therapy occurs within 72 hrs. The maintenance therapy with 0.2mg/day is adequate. Diet with high folate content is recommended.

Vitamin B12 deficiency anemia

Vitamin B12 is a water soluble vitamin comprising of biologically active cobalamins; methylcobalamin and adenosylcobalamin. They are essential for methylation of homocysteine to methionine and conversion of methyl malonyl CoA to succinyl CoA. They are critical to DNA, RNA and protein synthesis. Rich source of vitamin B12 are animal proteins i.e, meat, egg and fish. Daily requirement is 0.4 to 2.4mcg.

Etiology

Anemia due to dietary deficiency of vitamin B12 is uncommon. It occurs in breast-fed infants due to the low

levels of B12 in breast milk from B12 deficient mothers and manifests during the first 6 to 18 months of age. Vitamin B12 deficiency can occur due to inadequate dietary intake of foods rich in B12, lack of the intrinsic factor, impaired intestinal absorption or absence of vitamin B12 transport protein. It is common in vegans.

Clinical manifestations

B12 deficiency often presents with non-specific symptoms such as weakness, lethargy, feeding difficulty, failure to thrive and irritability. Pallor and glossitis are commonly seen. B12 deficiency affects the brain development during infancy.[10] Neurologic features such as hypotonia, developmental delay, seizures, sensory deficit and neuropsychiatric changes can occur in the absence of hematologic abnormalities.[10]

Laboratory diagnosis

The peripheral blood picture is similar to folate deficiency presenting with macrocytic anemia. The bone marrow is cellular with megaloblastic features. Serum vitamin B12 levels are low. The serum methylmalonic acid and homocysteine levels are elevated in B12 deficiency while in folate deficiency only serum homocysteine is increased.[11] Excessive excretion of methylmalonic acid in the urine is a sensitive and reliable index of B-12 deficiency.

Treatment

Recent data suggest oral B12 supplement is as effective as parenteral in nutritional deficiency.[12] Treatment with oral B12 deficiency is 0.2mcg/kg daily for two days followed by 1000 mcg/day for 2-7 days, then 100mcg/day for 2-7 days, 100mcg/week for 1 month. For the maintanence therapy 100mcg monthly once is given either IM/SC.[12, 13]

Vitamin A deficiency anemia

Vitamin A deficiency causes anemia, but lacks complete characterization as a clinical entity. Vitamin A enhances the growth and differentiation of erythroid progenitor cells and potentiates immunity to infection.[14] Vitamin A deficiency affects the mobilization of iron stores from the tissues, making iron unavailable for hemoglobin synthesis. Improving the vitamin A status reduces anemia. However, further work up is needed for confirmation.

Vitamin C deficiency anemia

Vitamin C enhances the absorption of iron and deficiency is associated with anemia and scurvy.

Vitamin E deficiency anemia

Vitamin E deficiency anemia is commonly seen in premature babies and occurs due to inadequate absorption of fat soluble vitamins and it presents as hemolytic anemia.

Conclusion

Nutritional anemia is a common public health problem and preventive measures like creating awareness about the importance of dietary factors, control of infections and infestations, early detection and specific treatment will reduce the morbidity and mortality.

Points to Remember

- ***Iron deficiency is the most common cause of nutritional anemia both as isolated or as combined deficiency.***
- ***Serum ferritin along with C-reactive protein serves as the best indicator of body iron stores.***
- ***Iron deficiency anemia is treated with oral iron supplements in appropriate form, dose and duration.***
- ***Folate and B12 deficiency during infancy have adverse impact on the developing brain.***
- ***Oral vitamin B 12 is as effective as parenteral B12.***

References

1. Mother and child nutrition in the tropics and subtropics; Nutritional Anemias. Chapter 9, J Trop Pediatr 339-355.
2. Gomber S, Bhawna, Madan N, Lal A, Kela K. Prevalence and etiology of nutritional anemia among school children of urban slums; Indian J Med Res 2003;118: 167 -171.
3. Koury MJ, Ponka P. New insights into erythropoiesis: The roles of folate, vitamin B-12 and iron. Annu Rev Nutr 2004; 24:105-131.
4. Canadian Paediatric Society Nutrition Committee. Iron needs of babies and children. Paediatr Child Health 2007; 12(4): 333–334.
5. Baker RD, Greer FR. Committee on Nutrition American Academy of Pediatrics. Diagnosis and prevention of iron deficiency and iron deficiency anemia in infants, and young children (0-3 years of age). Pediatrics 2010; 126:1040-1050.
6. Lozoff B, Jimeneze E, Hagen J, Mollen E, Wolf AW. Poorer behaviour and development outcome more than 10yrs after treatment for iron deficiency in infancy. Pediatrics 2000;105: e51.
7. Lukowski AF, Koss M, Burden MJ, Jonides J, Nelson CA, Kaciroti N, et al. Iron deficiency in infancy and neuro cognitive functioning at 19 yrs: evidence of long term deficits in executive function and recognition memory. Nutr Neurosci 2010;13: 54-70.

8. Wayne Thomas D, Rod f.Hinchliffe, Carol Briggs, Iain C.Macdougall,Tim Littlewood andIvor cavill-British committeefor standards in Haematology. Guidelines for the laboratory diagnosis of functional iron deficiency. Br J Haematol 2013;161:639 -648.
9. Yewale VN, Devan B. Treatment of iron deficiency anemia in children: a comparative study of ferrous ascorbate and colloidal iron. Indian J Pediatr 2013; 80: 385-390.
10. Crary SE, Hall K, Buchanan GR. Intravenous iron sucrose for children with iron deficiency failing to respond to oral iron therapy. Pediatr Blood Cancer 2011; 56: 615-619.
11. Black MM. Effect of B12 and folate deficiency on brain development in children. Food Nutr Bull 2008; 29: S126-S131.
12. JamesHarper,MD; Pediatric Megaloblastic Anemia updated 2015. http://emedicine.medscape.com/article/959918-overview
13. Rasmussen SA, Fernhoff PM,, Scanlon KS, Vitamin B12 deficiency in children and adolescents. J Pediatr 2001;138:10-17
14. Semba RD, Bloem MW. The anemia of vitamin A deficiency: epidemiology and pathogenesis. Eur J Clin Nutr 2002;56: 271-281.

CLIPPINGS

Nasogastric feeding tubes from a neonatal department yield high concentrations of potentially pathogenic bacteria- even one day after insertion.

Preterm infants are vulnerable to pathogens and at risk of developing necrotizing enterocolitis (NEC) or sepsis. Nasogastric feeding tubes (NG-tubes) might contaminate feeds given through them due to biofilm formation.

This was an observational study of used NG-tubes from a tertiary neonatal department. After removal, the NG tube was flushed with a 1-ml saline solution to determine the density of bacteria by culture and relate it to the duration of use and any probiotic administration through the tube.

Out of the 94 NG-tubes, 89% yielded more than 1,000 colony-forming units (CFU)/ml bacteria and 55% yielded the potentially pathogenic Enterobacteriaceae and/or Staphylococcus aureus. The mean concentration in the yield was 5.3 (SD: 2.1, maximum 9.4) log10CFU/ml. Neither the presence of contamination nor the density was associated with the time the NG-tube had been in use. Probiotic administration did not protect against contamination.The study concluded that NG-tubes yield high densities of bacteria even within the first day of use. Further studies are needed to determine if changing the NG-tubes between meals or once a day will make a positive impact on tube contamination and clinical parameters.

Petersen SM, Greisen G, Krogfelt KA. Nasogastric feeding tubes from a neonatal department yield high concentrations of potentially pathogenic bacteria- even 1 d after insertion. Pediatr Res. 2016 Sep;80(3): 395-400. doi: 10.1038/pr.2016.86. Epub 2016 Apr 11.

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HEMATO ONCOLOGY

PREVENTIVE STRATEGIES FOR THALASSEMIA

***Anupam Sachdeva**
****Arun S Danewa**

Abstract: *Thalassemias are group of autosomal recessive disorders of hemoglobin chain production. This inherited disorder requires life-long management in the form of regular blood transfusions and chelation therapy imposing a great burden on the family as well as the country. Various strategies for thalassemia prevention including genetic counselling, carrier detection and prenatal diagnosis have decreased the burden of disease but there is a need to increase awareness about the preventive strategies with special focus on pregnant women.*

Keywords: *Prenatal diagnosis, Preventive strategies, Thalassemia*

The thalassemias are a group of autosomal recessive disorders caused by reduction or absent production of one or more of the globin chains that make up the hemoglobin (Hb) tetramers. Over the years, our knowledge about thalassemia has expanded including its genetic basis, disease modifiers, preventive strategies including carrier detection by high performance liquid chromatography (HPLC), prenatal diagnostic methods and non-invasive methods including fetal DNA extraction from maternal blood. Despite all these, thalassemia remains to be a significant public health problem and poses an economic burden to the country as well.

Need for thalassemia prevention

Thalassemias are among the most commonly inherited diseases worldwide, affecting individuals originating from the Mediterranean area, Middle East, Central Asia, Indian subcontinent and Southeast Asia.[1] Worldwide, more than 240 million individuals are carriers of hemoglobinopathies including β-thalassemia. In India, the mean prevalence is around 3.3%.[2] Every year approximately 100,000 children with thalassemia major are born world over, of whom 10,000 are born in India.[3]

Management of thalassemia is life-long in the form of regular 3 weekly filtered packed red cell transfusions, chelation therapy for iron overload and management of complications associated with iron overload and transfusions, including osteoporosis, cardiac dysfunction, endocrine problems, infections due to hepatitis B and C, HIV and CMV. Despite treatment, the overall life expectancy of these patients is less due to unavoidable treatment related complications. All these impose a huge economic burden on the family as well as the country. In India, it has been estimated that the total cost for treating thalassemia major disease is about 2,10,000 INR / child / year, which is beyond the reach of the majority of families. India being a developing country with limited resources, priority tends to be given to tackling high rates of infant and child mortality from infections, other diseases and malnutrition.[3] As a result, hereditary disorders receive little attention. In this scenario, preventive strategies are the most important and cost-effective public health measures to reduce the burden of the disease.

Strategies for thalassemia prevention

Various preventive strategies for thalassemia prevention include genetic counseling, carrier detection and prenatal diagnosis using invasive as well as non-invasive techniques.

A. Genetic counseling

Genetic counseling is an important yet less commonly applied strategy for thalassemia prevention. As per international rules and regulations, genetic counseling should be performed in a nondirective way by the pediatrician trained in genetics. The high risk couples should be counseled with adequate information about the disease to guide them in appropriate decision making about reproduction. The discussion should include various options available such as birth control, mate selection, adoption, fetal testing including prenatal diagnosis and preimplantation diagnosis, or artificial insemination by

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normal donors as well as the details of the natural history of the disease. HLA typing on fetal DNA may be advised, to check whether the HLA typing of the fetus is identical to that of a previous unaffected sibling. This helps in identifying a suitable bone marrow donor so that the decision about continuation of pregnancy can be taken if the genotype is inconclusive and phenotype turns out to be diseased.[4] This method also helps in inductive screening wherein, the counselee is informed about the risk for their relatives and getting them investigated for carrier state if possible as reported in Pakistani populations.[5]

B. Carrier screening

Who should be screened?

In the late 1970s, pilot population programs directed to prevent β-thalassemia major by carrier screening, counseling and prenatal diagnosis were started in several at-risk populations in the Mediterranean area (Cyprus, Sardinia, several regions of Continental Italy, and Greece).[6-8]

The major target groups included pregnant women, relatives of the affected, high school or college students or the community at large. Different countries focused on different groups including general population screening at school age, as reported in Thailand; premarital screening, as described in middle east countries such as north Cyprus, where abortion is not generally accepted; and the screening of couples at marriage or in early pregnancy, as performed in Sardinia.[9] Cascade screening, a method of screening wherein siblings and relatives of affected child are screened has been found to be highly effective in identifying carriers in Pakistan.[5] In our country, most of the comprehensive programs involving carrier detections, molecular diagnostics, genetic counseling and prenatal diagnosis primarily focus on pregnant women.

How should the carriers be identified?

The technique to be used should be affordable, applicable and accurate. Various diagnostic methods for screening include laboratory techniques like 'naked eye single tube red cell osmotic fragility test' (NESTROFT), RBC indices including MCV and MCH and HPLC analysis of hemoglobins. It is recommended that in India, the primary strategy for carrier screening should be NESTROFT along with red cell indices, followed by confirmation with HPLC. In view of large numbers of subjects requiring screening in India, the better option would be to use electronic cell counters for measuring red cell indices including MCV and MCHC. Those individuals who have an MCV<78fl and an MCHC<27pg should undergo HPLC for HbA2 detection which is the most important feature for identifying heterozygous β-thalassemia.[10] In rural areas, NESTROFT may be used for initial analysis if electronic cell counters are not available as NESTROFT has a sensitivity of over 95% and a negative result picks out the normal very well.[11,12] HbA2 level between 3.5%-7% is suggestive of carrier state. However, a critical problem in β-thalassemia carrier screening is the identification of silent β-thalassemia or the triple-quadruple α-gene arrangement, which by interacting with typical β-thalassemia may result in the clinical features of mild or severe β-thalassemia. Silent β-thalassemia (normal HbA2 β-thalassemia type 1) shows normal hematological features and HbA2 level and may be identified solely by globin chain synthesis analysis or β-globin gene analysis.[13,14] As β-thalassemias display a marked heterogeneity at the molecular level with more than 200 different mutations described so far, the ultimate confirmatory diagnosis relies upon molecular procedures which are PCR-based like 'reverse dot blot (RDB) allele-specific oligonucleotide (ASO) hybridization' analysis and allele-specific amplification [amplification refractory mutation system (ARMS)].[15]

C. Prenatal diagnosis

Prenatal diagnosis should be advised in all pregnancies where both partners are carriers of β-thalassemia and thus are at risk of having a fetus with thalassemia major. Most commonly used techniques include obstetrical invasive procedures like chorionic villus sampling (CVS), amniocentesis and fetal cord blood sampling. Earliest isolation of fetal DNA can be done by CVS at 10-12 weeks of gestation where fetal chorionic villi (at least 30 mg) is obtained transabdominally or transcervically under ultrasound guidance. If performed earlier, it may result in fetal limb anomalies. This technique has been chosen by many centres because of the advantage of being used within the first trimester and a low risk (<1%) of fetal loss. Amniocentesis, the second most commonly used technique for prenatal diagnosis uses amniotic fluid for fetal DNA extraction and is usually performed within 16-18 weeks of gestation. It has the advantage of easier technique but relatively increased risk of abortion, risk of inadequate fetal cell extraction with greater chances of contamination with maternal cells compared to CVS. Couples presenting after 20-22 weeks of gestation are taken for fetal cord blood sampling where the associated risk of abortion is 1%-2%. Fetal DNA thus isolated by any of these methods is analyzed with one of the methods described for the detection of known mutations in the process of carrier identification. Prenatal diagnosis by DNA analysis is today

available in many at-risk populations, including those from Hong Kong, Taiwan, China and India, in addition to those from the Mediterranean area, Northern Europe and North and South America. Misdiagnosis may occur for several reasons i.e., failure to amplify the DNA fragment, mispaternity, maternal contamination and sample exchange.

Preimplantation genetic diagnosis is performed either by biopsy of one to two blastomeres in eight-cell embryos after in vitro fertilization (by intracytoplasmatic sperm injection) or by biopsy of trophectoderm cells from blastocyst.[16] Preconceptional diagnosis is based on the analysis of the first polar body of unfertilized eggs followed by analysis of the second polar bodies after fertilization, which is performed to avoid misdiagnosis resulting from recombination during the first meiosis.[17] Currently, to avoid the complications of invasive procedures, non-invasive procedures like analysis of fetal cells in maternal circulation have been made.[18,19] However, the complexity of the cell isolation process and the lack of reproducibility preclude the use of this approach in clinical practice.

Thalassemia prevention - Global experience

Preventive programmes carried out based on heterozygous detection and counselling to avoid marriage between carriers, without prenatal diagnosis, have not been very effective around the world in comparison to those which include fetal diagnosis as an additional preventive strategy as shown in Sardinia, Cyprus, Greece and Italy.[20] Pre-marriage screening was used at Iran in 1995 wherein all couples wanting to marry are required to be checked for carrier status of thalassemia for receiving permit for marriage registration. The male partner is first tested by blood cell indices followed by screening of women, in case he is a carrier as suggested by red cell indices. Hemoglobin A2 (HbA2) analysis is performed in at risk couples followed by counseling if both partners are carriers. In a study done in Iran over a period of 5 years (1997-2001), 2.7 million couples were tested, of whom 10,290 were at risk.[21] Of these, 53% decided to get married, 29% separated, and 18% were uncertain. But prenatal diagnosis is one step ahead of this method if available. Cascade or inductive screening identifies many more carriers than general population screening, as was shown in a study in Pakistan.[5]

Thalassemia prevention - Indian experience

The largest programme of screening for thalassemia in the population has been carried out in Gujarat by the Indian Red Cross Society in Ahmedabad and other cities where 370,117 subjects were screened for carrier status using HPLC system, which included 173,112 students, 45,000 youths and 8,377 pregnant women from 2004-2010 and results revealed that carrier rate varied from 4.3 to 5.0 per cent.[22] Another important project carried out under the Prime Minister's Jai Vigyan Thalassemia Control Programme was in six cities in India with a high prevalence of hemoglobinopathies including Mumbai (Maharashtra), Vadodora (Gujarat), Dibrugarh (Assam), Kolkata (West Bengal), Ludhiana (Punjab) and Bangalore (Karnataka).[23,24] Around 29,898 college students and 26,916 pregnant women were screened and the prevalence of β-thalassemia trait varied from 1.5% to 3.4% among college students and 1.3% to 4.2% among pregnant women. Most of the carriers were observed among certain communities such as vellalas, sindhis, aroras, lohanas, mandls, pillais, jains, khatirs and baidyas. This study came up with certain essential recommendations like broadcasting of multimedia awareness programmes, establishment of adequate number of centres for testing, combination of NESTROFT (osmotic fragility) test along with RBC indices including MCH and MCV and finally estimation of HbA2 by HPLC which is the most accurate method for diagnosis of β-thalassemia heterozygotes. HPLC would be recommended in all those samples that are NESTROFT +ve or have MCV less than 80 fl, or MCH less than 27 pg/cell.[23] Another target population i.e., high school or college students were screened by Colah and colleagues to include 5682 school children (age 11-18 yr) from 75 schools in Mumbai city between 1984 and 1988 of which, 153 (2.7%) were found to be β-thalassemia heterozygotes but after 20 years it was possible to locate only 71 of the 153 children who were carriers of β-thalassemia. Only 47 of these 71 families could be contacted. Only 12 of the 47 individuals contacted (26%) recollected that they were β-thalassemia carriers. None of the 41 individuals who were now married had revealed their carrier status or had their partners tested before marriage.[25] Tamhankar, et al carried out premarital testing for thalassemia carrier state in three groups: extended family members of diagnosed cases of thalassemia/ haemoglobinopathies, unmarried adult cases of anemia attending the hospitals' outpatient department and adult college students. As much as 99% of prospective carrier couples married even after knowing their high-risk status and opted for prenatal diagnosis.[26]

In India, All India Institute of Medical Sciences, New Delhi was the first to start prenatal diagnosis in 1996 for 415 cases. This was followed by Christian Medical College, Vellore[27] and National Institute of Immunohematology and Wadia Hospital for Children, Mumbai.[28] Currently prenatal diagnosis is available in the centres listed in Table I and in a few commercial laboratories.[29]

Table I. Centres in India for prenatal diagnosis of thalassemia

Place	Centre
New Delhi	Sir Ganga Ram hospital, All India Institute of Medical Sciences
Mumbai	National Institute of Immunohaematology and Wadia Hospital for Children
Vellore	Christian Medical College and Hospital
Lucknow	Sanjay Gandhi Postgraduate Institute of Medical Sciences
Hyderabad	Center for DNA Fingerprinting and Diagnostics, Center for Cellular and Molecular Biology
Chandigarh	Postgraduate Institute of Medical Education and Research

Future strategies for preventing thalassemia

The conclusion drawn from the review of literature suggests that future strategies should have less focus on premarital screening. The policy should determine the carrier status of subjects during early pregnancy, so that prenatal diagnosis can be obtained in the at risk couples. Strategy for screening programmes for β-thalassemia should be to sensitize the community to the problem, establish hematological technologies for screening and molecular and obstetric techniques for prenatal diagnosis.

Points to Remember

- ***Thalassemias are a group of autosomal recessive disorders with defective or absent hemoglobin chain synthesis.***
- ***Management includes life-long packed red cell transfusions, chelation therapy and management of complications with iron overload.***
- ***Prenatal diagnosis should be advised when both partners are carriers of β-thalassemia which includes chorionic villus sampling, amniocentesis and fetal cord blood sampling.***
- ***More efforts are needed to increase awareness about the preventive strategies.***

References

1. Winichagoon P, Saechan V, Sripanich R, Nopparatana C, Kanokpongsakdi S, Maggio A. Prenatal diagnosis of β-thalassemia by reverse dot blot hybridization. Prenatal Diagn 1999; 19:428–435.
2. Weathrall DJ, Clegg JB. Inherited Haemoglobin Disorders: an increasing global problem. Bull World Health Organ 2001;79:704-712.
3. Verma IC, Choudhry VP, Jain PK. Prevention of thalassemia: A necessity in India. Indian J Pediatr 1992; 59: 649-654.
4. Orofino MG, Argiolu F, Sanna MA, Rosatelli MC, Tuveri T, Scalas MT, et al. Fetal HLA typing in β-thalassemia: Implications for hemopoietic stem-cell transplantation. Lancet 2003; 362: 41–42.
5. Ahmed S, Saleem M, Modell B, Petrou M. Screening extended families for genetic hemoglobin disorders in Pakistan. N Engl J Med 2002; 347: 1162–1168.
6. Angastiniotis MA, Hadjiminas MG. Prevention of thalassemia in Cyprus. Lancet 1981; 1:369–371.
7. Cao A, Rosatelli MC, Galanello R. Control of β-thalassemia by carrier screening, genetic counselling and prenatal diagnosis: The Sardinian experience. Ciba Found Symp 1996;197:137–155.
8. Loukopoulos D. Current status of thalassemia and the sickle cell syndromes in Greece. Semin Hematol 1996;33:76–86.
9. Koren A, Profeta L, Zalman L, Palmor H, Levin C, Zamir RB, et al. Prevention of â Thalassemia in Northern Israel - a Cost-Benefit Analysis. Mediterr J Hematol Infect Dis 2014;6:e2014012. doi: 10.4084/MJHID.2014.012. eCollection 2014.
10. Weatherall DJ, Clegg JB. The thalassemia syndromes. 4th edn, Blackwell Science, Oxford., UK, 2001.
11. Mehta BC, Iyer PD, Gandhi SG, Ramnath SR, Patel JC. Diagnosis of heterozygous beta-thalassemia in a population with high prevalence of iron deficiency. Indian J Med Sci 1973;27:832-835.
12. Gomber S, Madan N. Validity of Nestroft in screening and diagnosis of β-thalassemia trait. J Trop Pediatr 1997;43:363-366.
13. Gonzalez-Redondo JM, Stoming TA, Kutlar A, Kutlar F, Lanclos KD, Howard EF, et al. A C!T substitution at nt 2101 in a conserved DNA sequence of the promotor region of the β-globin gene is associated with “silent” β-thalassemia. Blood 1989;73:1705–1711.
14. Galanello R, Barella S, Ideo A, Gasperini D, Rosatelli C, Paderi L, et al. Genotype of subjects with borderline hemoglobin A2 levels: Implication for β-thalassemia carrier screening. Am J Hematol 1994;46:79–81.

15. Saiki RK, Walsh PS, Levenson CH, Erlich HA. Genetic analysis of amplified DNA with immobilized sequence specific oligonucleotide probes. Proc Natl Acad Sci 1989;86: 6230–6234.
16. Kokkali G, Synodinos JT, Vrettou C, Stavrou D, Jones GM, Cram DS, et al Blastocyst biopsy versus cleavage stage biopsy and blastocyst transfer for preimplantation genetic diagnosis of β-thalassemia: A pilot study. Hum Reprod 2007;22:1443–1449.
17. Verlinsky Y, Ginsberg N, Lifchez A, Valle J, Moise J, Strom CM. Analysis of the first polar body: Preconception genetic diagnosis. Hum Reprod 1990;5:826–829.
18. Cheung MC, Goldberg JD, Kan YW. Prenatal diagnosis of sickle cell anaemia and thalassemia by analysis of fetal cells in maternal blood. Nat Genet 1996;14:264–268.
19. Bianchi DW, Williams JM, Sullivan LM, Hanson FW, Klinger KW, Shuber AP. PCR quantitation of fetal cells in maternal blood in normal and aneuploid pregnancies. Am J Hum Genet 1997;61:822–829.
20. Cao A, Galanello R, Rosatelli MC. Prenatal diagnosis and screening of the haemoglobinopathies. Baillieres Clin Haematol 1998;11:215-238.
21. Samavat A, Modell B. Iranian national thalassemia screening programme. BMJ 2004; 329:1134-1137.
22. Indian Red Cross Society (IRCS), Gujarat State Branch. Annual Report 2009-2010. Ahmedabad: IRCS, Gujarat State Branch; 2010.
23. Mohanty D, Colah R, Gorakshakar A, editors. Report of the Jai Vigyan S & T Mission Project on community control of thalassaemia syndromes - Awareness, screening, genetic counselling and prevention. A National Multicentric Task Force Study of Indian Council of Medical Research-New Delhi, 2008. New Delhi, ICMR, 2008.
24. Colah R, Surve R, Wadia M, Solanki P, Mayekar P, Thomas M, et al. Carrier screening for beta-thalassemia during pregnancy in India: a 7-year evaluation. Genet Test 2008;12: 181-185.
25. Colah R, Thomas M, Mayekar P. Assessing the impact of screening and counselling high school children for β-thalassemia in India. J Med Screen 2007;14:158.
26. Tamhankar PM, Agarwal S, Arya V, Kumar R, Gupta UR, Agarwal SS. Prevention of homozygous beta thalassemia by premarital screening and prenatal diagnosis in India. Prenat Diagn 2009;29:83-88.
27. Muralitharan S, Srivastava A, Shaji RV, Mathai M, Srivastava VM, Dennison D, et al. Prenatal diagnosis of beta-thalassemia mutations using the reverse dot blot technique. Natl Med J India 1996;9:70-71.
28. Thakur (Mahadik) C, Vaz F, Banerjee M, Kapadia C, Natrajan PG, Yagnik H, et al. Prenatal diagnosis of beta-thalassemia and other haemoglobinopathies in India. Prenat Diagn 2000;20:194-201.
29. Saxena R, Jain PK, Thomas E, Verma IC. Prenatal diagnosis of beta-thalassemia: experience in a developing country. Prenat Diagn 1998;18:1-7.

NEWS AND NOTES

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HEMATO ONCOLOGY

APPROACH TO A BLEEDING CHILD

***Nitin Shah**

Abstract: *Hemostasis is a perfect balance between fluidity of flowing blood on one hand and clotting when required on other hand. Vessel wall, platelets, coagulation factors and their regulators as well as fibrinolytic processes play a role in this balance. While approaching a child with bleeding, systematic approach starting with clinical history, detailed examination, screening laboratory tests and at the end confirmatory test is essential. Clinical clues also at times help to clinch the diagnosis. Newer laboratory tests have helped further diagnosis of rare bleeding disorders.*

Keywords: *Bleeding child, Approach, Screening tests, Confirmatory tests*

It is amazing that blood keeps flowing through the smallest capillaries throughout the life without clotting and yet when there is an injury it clots effectively and rapidly to prevent major blood loss followed by timely dissolution of the clot formed. This delicate balance is achieved through vascular factors, platelets and plasma proteins like coagulation factors, their inhibitors and fibrinolytic factors working synchronously.

Goals while approaching a child with bleeding are to ascertain severity of bleeding so as to offer immediate measures to stop the bleeding and once stable to find out the cause of bleeding and decide on definitive treatment options. This is achieved by thorough clinical history, head to toe physical examination followed by screening laboratory tests and lastly specific tests to confirm the diagnosis.[1]

Four questions that one should address while faced with a bleeding child are: 1) Is the bleeding significant? 2) Is it a local cause or a systemic cause? 3) Is it inherited or acquired in nature? 4) Is it due to vascular or platelet or coagulation abnormality? Or is it multifactorial in nature?[1] Points to be considered while addressing these questions include age of onset of first episode of bleeding, sex of the child, type of bleeding, site of bleeding, provocation of bleeding episode, past history of bleeding and family history of similar bleeding.

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1) Is the bleeding significant?

Bleeding would occur following trauma but generally stops in 1-3 minutes especially with pressure. One should think of a bleeding disorder when the bleeding occurs spontaneously, does not stop on pressure, recurs later, leads to significant blood loss or there are systemic clues or positive family history.

2) Is it a local cause or a systemic cause?

There are several local causes of bleeding depending on the site of bleeding like epistaxis due to nose picking, bleeding from Little's area, nasal foreign body, vigorous blowing of nose during rhinitis and dry nasal mucosa; lower gastrointestinal bleed due to polyps, rectal fissure; umbilical bleeding due to slipped ligature, umbilical granuloma; oral bleeding due to poor dental hygiene, tongue bite, etc. Bleed due to local cause is from one site and recurs from the same site, recurring from the same site without other systemic clues, positive family history with the laboratory screening tests most often being normal.[1]

3) Is it inherited or acquired in nature?

Inherited bleeding disorders start very early in life (mild hemophilia is an exception)[2] and there is history of bleeding from umbilical cord, huge cephalhematoma, excessive bleeding when the primary teeth fall, bleeding after minor surgery like circumcision or joint bleeds etc. Often there is positive family history of similar bleeding. Hemophilia A, Hemophilia B and Wiskott Aldrich syndrome are X-linked recessive disorders with history of bleeding in males on maternal side. Other coagulation factors deficiency are autosomal recessive disorders with history of bleeding in both the sexes in siblings or cousins and consanguinity in family. Joint bleeding, muscle bleeding and deep seated ecchymosis are hallmarks of hemophilia. Von Wille-brand disease, some types of

qualitative platelet defects, dysfibrinogenemia and hereditary hemorrhagic telangiectasia are examples of autosomal dominant conditions with history of bleeding in parents and grandparents suggesting vertical transmission and may have variable penetrance and skipped generations.

Acquired disorders start late in life (Vit K deficiency associated hemorrhagic disease of newborn is an exception). There would be no evidence of bleeding in the past even with some surgeries and no family history of similar bleeding. There may be some systemic illness like liver or renal disease or history of drugs taken like NSAIDs to suggest the cause of bleeding. Examples of systemic disease leading to bleeding include liver disease leading to factor II, VII, IX and X deficiency or platelet dysfunction; renal diseases leading to low platelets like in hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP) or platelet dysfunction as seen in renal failure; malabsorption syndromes leading to vitamin K deficiency; acute promyelocytic leukemia (APML) with associated DIC, bone marrow diseases leading low platelets; systemic vascular diseases like SLE with associated low platelets and anti-cardiolipin antibody syndrome / anti phospholipid antibody (ACLA/APLA syndrome).

Certain syndromes are associated with bleeding and are easy to be picked up. Hereditary telangiectasia is associated with similar lesions in oral mucosa or nasal mucosa with local bleeding.[3] Pigment disorder with partial albinism like Hermansky Pudlak syndrome is associated with platelet dysfunction and bleeding.[4] Thrombocytopenia with absent Radius syndrome (TAR) is associated with bleeding due to low platelets. Factor V deficiency is associated with syndactyly.[4] Keloids may be seen in patients with factor XIII deficiency or fibrinogen deficiency. Recurrent ecchymosis and poor scars are seen in patients with Ehler Danlos syndrome which will also have hyperextensible joints and cutis elastica.[5] Patient with Wiskott Aldrich syndrome will present with recurrent infections, eczema and bleeding due to small platelets.[4] Children with Kasabach Merritt syndrome will have giant hemangioma and bleeding due to low platelets following localized DIC.

4) Is it due to vascular or platelet or coagulation abnormality? Or is it multifactorial in nature?

Vascular or platelet type of bleeding will manifest with pinpoint petechiae or small superficial ecchymosis and mainly mucosal bleeds like epistaxis or menorrhagia. The bleeding that starts immediately following trauma is easily controlled by local pressure and does not recur later. Exsanguinating bleeds are rare except GI bleeds. Commonest cause of platelet type of bleeding in children is immune thrombocytopenic purpura (ITP) which is acquired in nature without family history.[6] Coagulation abnormality presents with huge deep seated ecchymosis, muscle bleeds and joint bleeds and the bleeding can be exsanguinating as seen in hemophilia.[2] There is a delay in the onset of bleeding after an injury, but is difficult to control with local pressure and tends to recur once pressure is released. The commonest cause of this type of bleeding is hemophilia and there is often history of bleeding from early age and a positive family history.

Typical clinical scenarios helps to narrow down the diagnosis. Newborn with excessive bleeding from umbilical stump could be due to factor XIII deficiency.[7] Newborn with intracranial bleeding may be due to factor XIII deficiency, fibrinogen defects or allo/auto immune thrombocytopenia or cholestatic liver disease. Excessive bleeding in infants and young children after circumcision is most likely to be due to hemophilia.[2] An older child with mainly skin bleeds following recent viral infection is most likely to be suffering from ITP.[6] A child who suffers from hemarthrosis after learning to walk is likely to be having hemophilia.[2] Lastly an adolescent girl with menorrhagia, recurrent epistaxis with positive family history in mother or father is most likely to have Von Willebrand disease.[8]

Laboratory evaluation

Screening tests are done in every child with bleeding disorder. Based on clinical evaluation and screening tests results, one needs to do further tests for confirmation to clinch the diagnosis and plan further treatment.

Screening tests

No single test can help to diagnose a case of bleeding disorder but at the same time there is no need to subject such a child to a battery of tests which are often not readily available and are universally expensive. Screening tests that one should do include complete blood count (CBC) and finger prick peripheral smear (PS), prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT) and serum fibrinogen level.[9] One may at times do bleeding time (BT) (by a controlled IVY method) especially in suspected platelet dysfunction disorders. BT should not be done in the presence of thrombocytopenia as it does not provide any additional information and can lead to bleeding from the cut and permanent scar formation. Clotting time and clot retraction

time have been given up world over as they lack sensitivity and specificity.

CBC, platelet count and peripheral smear (PS)

Hb, WBC count, absolute neutrophil count and platelet count helps to determine bone marrow status. Pancytopenia or bicytopenia is seen in bone marrow disease, whereas isolated thrombocytopenia will be seen in ITP. Peripheral smear helps in assessing platelets number, size and shape. Presence of 10-15 platelets per high power field suggests normal platelet count and is more reliable than automated machine report and helps rule out pseudothrombocytopenia which is due to in vitro platelet clumping in EDTA bulb due to preformed antibodies to EDTA and can be further confirmed by doing platelet count in phase contrast microscopy of platelet count in a citrated bulb which will be normal.[10] Presence of clumps of platelets suggests normal and functioning platelets. Absence of clumps suggests platelet dysfunction like seen in Glanzman's thrombasthenia. Large platelets are seen in regenerative thrombocytopenia like ITP.Thrombocytopenia with very large platelets suggest Bernard Soulier syndrome. Tiny platelets are seen in Wiskott Aldrich syndrome. Pale looking platelets are seen in Gray platelet syndrome.

PT and aPTT

aPTT is a screening test for extrinsic pathway and PT for intrinsic pathway. aPTT is normally ± 10 seconds of control. PT is normally ± 3 seconds of control. PT is better interpreted as INR which should be below 1.2. Both these tests are highly sensitive to temperature and hence should be performed as soon as possible after sample collection.[11] If there is going to be a delay in performing the tests, it is better to extract plasma and keep it in fridge till tests are performed. PT alone is prolonged in factor VII deficiency. aPTT alone is prolonged in factor VIII, IX or XI deficiency.

Table I. Screening tests and their interpretation

Screening laboratory tests in selected hemostatic disorders

Clinical bleeding	aPTT	PT	TCT	Bleeding time	Platelet count	Possible defects
Absent	Abnormal	Normal	Normal	Normal	Normal	High-molecular-weight kininogen, prekallikrein, Factor XII, lupus inhibitor
Present	Abnormal	Normal	Normal	Normal	Normal	XI, IX, VIII
Present	Abnormal	Abnormal	Normal	Normal	Normal	V, X, II, coumarin, Vitamin K deficiency, mild hepatic disease
Present	Normal	Abnormal	Normal	Normal	Normal	VII
Present	Abnormal	Normal	Normal	Abnormal	Normal	Von Willebrand disease
Present	Abnormal	Abnormal	Abnormal	Abnormal	Normal	Afibrinogenemia
Present	Normal	Normal	Normal	Abnormal	Abnormal	Thrombocytopenia
Present	Normal	Normal	Normal	Abnormal	Normal	Qualitative platelet disorder (aspirin, thrombasthenia, Bernard-Soulier syndrome)
Present	Normal	Normal	Normal	Normal	Normal	Factor XIII
Present	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal	Disseminated intravascular coagulation, severe liver disease.
Variable	Normal	Normal	Abnormal	Normal	Normal	Dysfibrinogenemia, myeloma, fibrinogen / fibrin degradation products
Present	Abnormal	Normal	Abnormal	Normal	Normal	Heparin

aPTT - partial thromboplastin time, PT - prothrombin time, TCT- thrombin clotting time

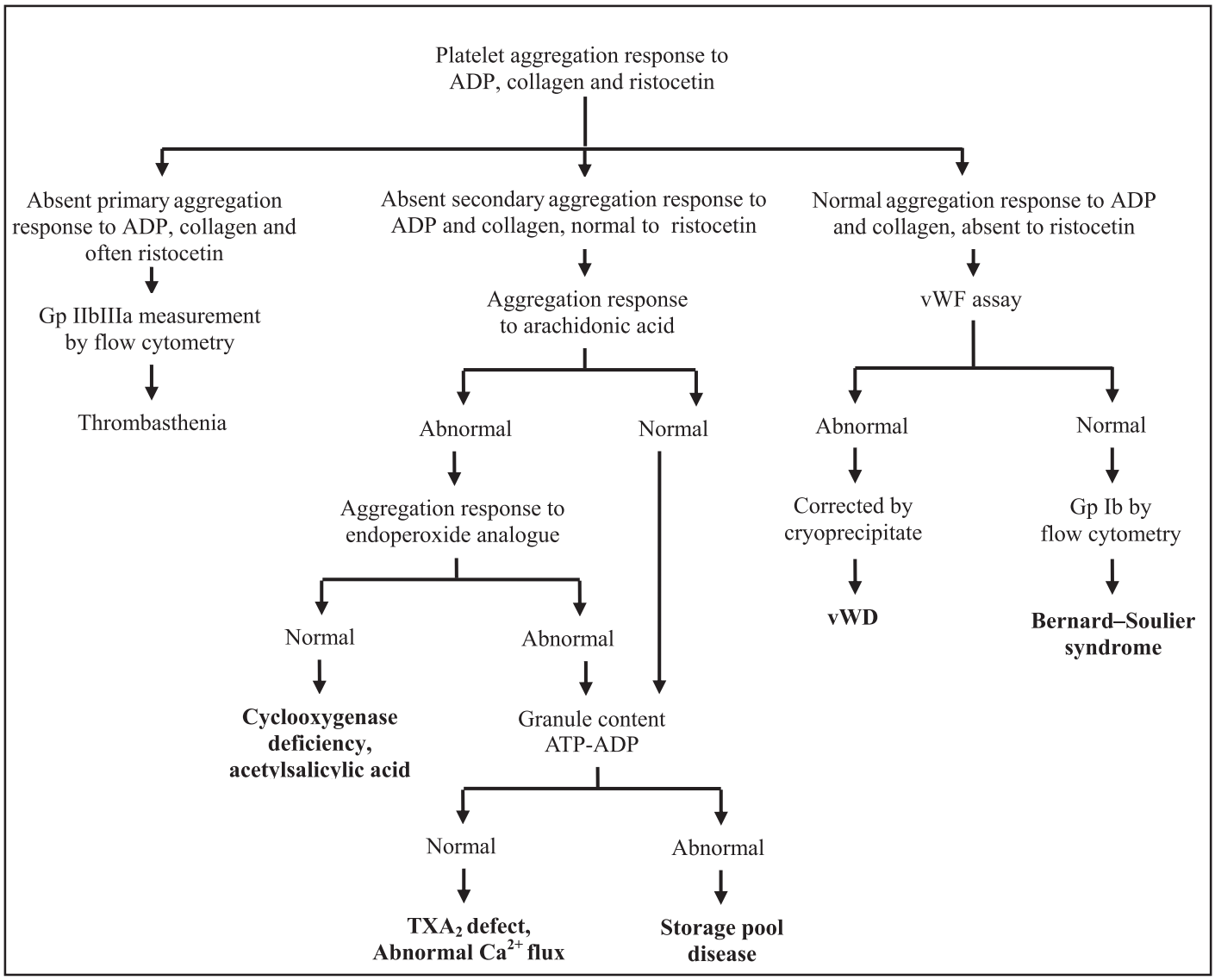

Fig.1. Platelet aggregation studies and their interpretation.

(TXA2 Thrombaxane A2, ADP adenosine diphosphate)

When both PT and aPTT are prolonged it suggests common pathway defect like factor V, II, X deficiency or multiple factor deficiency classically seen in vitaminK deficiency or liver disease with deficiency of factors II, VII, IX and X. aPTT in vitro is also prolonged in presence of inhibitors like lupus inhibitor as seen in SLE or APLA syndrome, but generally these patients have in vivo thrombo-embolism and not bleeding.[12] One must do mixing studies where aPTT is run again on a mixture of one mL of patient's plasma and one mL of pooled normal plasma as it will differentiate between deficiency of factors versus presence of inhibitor as the cause of prolonged aPTT. If there is factor deficiency, addition of pooled normal plasma will correct the prolonged aPTT by at least 50% whereas if there is presence of inhibitor, aPTT will not be corrected and in contrary will further prolong on incubation for four hours.

Thrombin time (TT) or Thrombin clotting time (TCT)

TCT measures thrombin induced conversion of fibrinogen to fibrin. Normal TCT is ± 3 seconds over control. Prolonged TCT will suggest either a/hypo/dys-fibrinogenemia. Simultaneous estimation of fibrinogen level by chemical methods will help further differentiate these conditions as prolonged TCT with absent or low fibrinogen levels will suggest a/hypo fibrinogenemia respectively whereas normal fibrinogen level will suggest dysfibrinogenemia. TCT can also be prolonged in the presence of inhibitors like heparin, myeloma proteins and fibrin degradation products which block either thrombin cleavage of fibrinopeptide or fibrin monomer polymerization.

Interpretation of screening tests is shown in Table I. Screening tests can be normal in a patient with bleeding like in factor XIII deficiency, mild factor deficiency, vascular purpura (like Henoch-Schonlein's purpura, Vasculitis, Ehler Danlos syndrome), battered baby syndrome, fictitious (or self-inflicted) purpura and scurvy.

Confirmatory tests

Based on the clinical clues and results of screening tests one can fairly narrow down the differentials and order for the specific confirmatory tests. This may include correction studies followed by factor assay in suspected case of factor deficiency, inhibitor assay if mixing studies show presence of inhibitor, bone marrow in a case of ITP or suspected leukemia or bone marrow failure syndrome, organ specific tests like liver function tests and renal function tests as required.

Factor assay: If PT alone is prolonged ask for factor VII assay. If aPTT alone is prolonged ask for factor VIII first as it is the commonest cause. If that is normal ask for factor IX and then factor XI in that order. If PT and aPTT both are prolonged ask for factor V or factor X or factor II. If TCT is prolonged ask for fibrinogen levels and interpret accordingly as discussed before. If all screening tests are normal ask for factor XIII by urea solubility assay. If aPTT is prolonged with normal factor VIII/IX/XI assay especially in a female and in presence of prolonged bleeding time think of Von Willebrand disease (vWD) and ask for vWF assay.

Platelet function studies and platelet receptor studies: In a patient with platelet type of bleeding clinically and normal platelet count suspect platelet dysfunction disorders especially if BT is prolonged and there is strong family history of similar bleeding. PS helps in many such cases to clinch a diagnosis as discussed before. One orders further platelet function studies commonly platelet aggregation studies using PFA100 or platelet aggregometer using various platelet agonists.[13] The interpretation of platelet aggregation studies is shown in Fig.1. Once platelet aggregation studies suggest a specific disorder, one can further confirm the same by doing platelet receptor analysis by flowcytometry which are available easily for Glanzman's disease and Bernard Soulier syndrome (BSS) that are the two commonest inherited platelet dysfunction disorders or electron microscopy for Hermansky Pudlak syndrome.[14,15]

Conclusions

In a child with a bleeding prob-lem, a careful history including family history, past history, detailed clinical examination, few important screening tests and then appropriate specialized tests will help in diagnosis and better management.

Points to remember

- ***Ascertain whether bleeding is due to local cause or systemic cause, inherited cause or acquired cause, vascular/platelet defect or coagulation defect.***
- ***Clinical clues at times help clinch the diagnosis in a syndromic child with bleeding.***
- ***Bleeding time is rarely required and clotting time is given up as a screening test.***
- ***CBC, PS, PT, aPTT and TCT form the screening tests in a bleeding child.***
- ***Prolonged aPTT can be also due to presence of inhibitors.***
- ***Normal screening tests for bleeding do not rule out bleeding disorders always.***
- ***Keep battered baby or fictitious purpura as a cause of bleeding in mind in a given clinical background.***

References

1. Khair K, Liesner R. Bruising and bleeding in infants and children-a practical approach. Br J Haematol 2006; 133:221-231.
2. Hoyer LW. Hemophilia A. N Engl J Med 1994;330: 38-47.
3. Haitjema T, Westermann CJ, Overtoom TT, Timmer R, Disch F, Mauser H, et al. Hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease): new insights in pathogenesis, complications, and treatment. Arch Intern Med 1996;156:714-719.
4. Warrier I, Lusher JM. Congenital thrombocytopenias. Curr Opin Hematol 1995; 2:395.
5. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. et al. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 1998;77:31-37.
6. Neunert C, Lim W, Crowther M, Cohen A, Lawrence Solberg Jr, Mark A. Crowther. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. Blood 2011;117:4190-4207.
7. Hsieh L, Nugent D. Factor XIII deficiency. Haemophilia 2008; 14:11901200.
8. Miller CH, Graham JB, Goldin LR, Elston RC. Genetics of classic Von Willebrand's disease. I. Phenotypic variation within families. Blood 1979;54:117-136.

9. Hillman C, Lusher JM. Tests of blood coagulation technical points of clinical relevance. In: Acquired Bleeding Disorders in Children, Lusher JM, Barhart MI (eds), Masson, New York, 1981;p107.
10. Payne BA, Pierre RV. Pseudothrombocytopenia: a laboratory artifact with potentially serious consequences. Mayo Clin Proc 1984;59:123-125.
11. Lowe GD, Forbes CB. Laboratory diagnosis of congenital coagulation defects. Clin Hematol 1979;8:79-94.
12. Lossing TS, Kasper CK, Feinstein DI. Detection of factor VIII inhibitors with the partial thromboplastin time. Blood 1977;49:793-797.
13. Mammen EF, Comp PC, Gosselin R, Greenberg C, Hoots WK, Kessler CM, et al. PFA-100 system: a new method for assessment of platelet dysfunction. Semin Thromb Hemost 1998;24:195-202.
14. Michelson AD. Flow cytometry: a clinical test of platelet function. Blood 1996;87:4925-4936.
15. Gahl WA, Brantly M, Kaiser-Kupfer MI, Iwata F, Hazelwood S, Shotelersuk V, et al. Genetic defects and clinical characteristics of patients with a form of oculocutaneous albinism (Hermansky-Pudlak syndrome). N Engl J Med 1998;338:1258-1264.

CLIPPINGS

Caffeine for the Treatment of Apnea in Bronchiolitis: A Randomized Trial.

The study was done to evaluate the efficacy and safety of caffeine citrate in the treatment of apnea in bronchiolitis.

Eligible infants aged ≤4 months presenting to the main pediatric emergency service with apnea associated bronchiolitis were stratified by gestational age (<34 weeks or longer) and randomized to receive a single dose of intravenous 25 mg/kg caffeine citrate or saline placebo. The primary efficacy outcome was a 24-hour apnea-free period beginning after completion of the blinded study drug infusion. Secondary outcomes were frequency of apnea by 24, 48, and 72 hours after study medication, need for noninvasive/invasive ventilation and length of stay in the hospital's pediatric intensive care/step-down unit.

A total of 90 infants diagnosed with viral bronchiolitis associated with apnea (median age, 38 days) were enrolled. The rate of respiratory virus panel positivity was similar in the 2 groups (78% for the placebo group vs 84% for the caffeine group). The frequency of apnea at 24 hours, 24-48 hours and 48-72 hours after enrollment and the need for noninvasive and invasive ventilation were similar in the 2 groups. No safety issues were reported.

The study concluded that a single dose of caffeine citrate did not significantly reduce apnea episodes associated with bronchiolitis.

Alansari K, Toaimah FH, Khalafalla H, El Tatawy LA, Davidson BL, Ahmed W. Caffeine for the Treatment of Apnea in Bronchiolitis: A Randomized Trial. J Pediatr. 2016;177: 204-211.

NEWS AND NOTES

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HEMATO ONCOLOGY

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Balasubramanian S

Abstract: *Hemophagocytic lymphohistiocytosis (HLH) is a life threatening illness often associated with malignancy, rheumatologic and infectious diseases. HLH presents with fever and involvement of many organ systems with hepatosplenomegaly, lymphadenopathy, rash and neurologic manifestations. Anemia and thrombocytopenia along with elevated ferritin, abnormal liver enzymes level and deranged coagulation profile should point towards HLH. Though hemophagocytosis on bone marrow examination is not seen in all cases, infiltration of the bone marrow by activated macrophages is consistent with the diagnosis. Immunological investigations are not essential for initiation of therapy. Treatment aim is to interrupt the amplification cascades of cytokines and suppress the hyperinflammation.*

Keywords: *Hemophagocytic lymphohistiocytosis, Children, Pathogenesis, Management*

Hemophagocytic lymphohistiocytosis (HLH), an uncommon systemic inflammatory clinical syndrome is associated with several conditions such as neoplastic, infectious, autoimmune or hereditary diseases. It was first described in 1952 by Farquhar and Claireaux.[1] It is a frequently fatal and under-diagnosed disease involving a final common pathway of hypercytokinemia leading to end-organ damage and death. Definitive diagnosis is challenging due to the lack of specificity of currently accepted diagnostic criteria and the absence of confirmatory gold standard.[1]

Classification

The primary form occurs in children with genetic abnormalities of the cytotoxic function of natural killer (NK) cells and T cells. The secondary form manifests at older ages in the presence of an associated infection, malignancy or autoimmune disease without an obviously identifiable genetic abnormality. However, these designations, primary and secondary have become less relevant because of absence of uniformity in presentation of these 2 groups. Currently genetic and acquired HLH are the more appropriate terminologies.

The primary HLH, often called familial hemophagocytic lymphohistiocytosis (FHL), refers to HLH caused by gene mutation at one of the FHL loci or in a gene responsible for an immunodeficiency syndrome. The secondary HLH often called sporadic or acquired HLH has been used to describe those without a known familial mutation and those for whom a clear trigger for HLH has been identified. However, this term may not be appropriate since many patients with secondary HLH may have a genetic defect associated with the syndrome and many children with primary HLH experience symptoms in response to one of the triggers, most commonly infections.[1]

The other terminologies often described in literature include virus-associated hemophagocytic syndrome, hemophagic histiocytosis and familial erythrophagocytic lymphohistiocytosis. Macrophage activation syndrome (MAS) is a form of HLH that occurs primarily in patient with juvenile idiopathic arthritis or other rheumatologic diseases often called reactive hemophagocytic syndrome.

Epidemiology

It is estimated that approximately 1 in 3000 children admitted to a hospital will have HLH. Infants are most commonly affected with the highest incidence in those <3 months. The male to female ratio is around 1:1 in children, whereas in adults there may be a slight male predisposition. Up to 25% of HLH cases have been reported to be familial.[2]

Pathogenesis

a. Immunopathogenesis

HLH is a syndrome of excessive inflammation and tissue destruction due to abnormal immune activation and is not a malignancy. This excessive inflammation is caused by a failure of normal down regulation of activated

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macrophages and lymphocytes. Macrophage activation results in secretion of excessive amounts of cytokines leading to severe tissue damage and organ failure.

NK cells eliminate damaged, stressed or infected host cells such as macrophages, typically in response to viral infection or malignancy. Cytotoxic lymphocytes (CTLs) are activated T lymphocytes that lyse autologous cells such as macrophages bearing foreign antigen associated with Class I histocompatibility proteins. In HLH, NK cells and/or CTLs fail to eliminate activated macrophages. Due to this lack of normal feedback regulation, excessive macrophage activity, highly elevated levels of interferon gamma and other cytokines occur. Majority of patients with HLH exhibit impaired cytotoxic function of NK cells and CTLs, in addition to excessive activation of macrophages.

Elimination of activated macrophages by NK cells and CTLs occurs through the process of perforin dependent cytotoxicity. NK cells and CTLs lyse target cells in a series of steps that include formation of an immunologic synapse, creation of a pore in the macrophage membrane and delivery of cytolytic granules into the macrophage. The granules contain a variety of proteases such as granzyme B (serine protease most commonly found in the granules of cytotoxic lymphocytes) that can initiate cell death, often through apoptosis. Most of the genetic defects in patients with familial HLH encode proteins involved in this process.

Toll-like receptor (TLR) activation of the immune system can be another cause of HLH. TLRs are nonantigen specific receptors on the surface of NK cells that are activated by components of bacteria, fungi, viruses or mycoplasma. Genes associated with TLR / interleukin 1 receptor (IL-1R) signalling are upregulated in patients with juvenile idiopathic arthritis and MAS.

Hemophagocytosis consists of the presence of red blood cells, platelets, white blood cells or their fragments within the cytoplasm of macrophages. Although its presence supports the diagnosis of HLH, hemophagocytosis by itself is neither pathognomonic nor required for the diagnosis.

Cytokine storm is due to the persistent activation of macrophages, NK cells and CTLs that occurs in patients with HLH leading on to excessive cytokine production. This is believed to be responsible for multiorgan failure and the high mortality. Cytokines found at extremely high levels in the plasma of patients with HLH include interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α), interleukins (IL) such as IL-6, IL-10, IL-12 and the soluble IL-2 receptor (CD25).[3-6]

The following immunodeficiency syndromes have been described in literature so far in association with HLH - Griscelli syndrome, Chediak-Higashi syndrome, X-linked lymphoproliferative disease, Interleukin-2-inducible T cell kinase (ITK) deficiency, CD27 (TNFRSF7) deficiency, Hermanski-Pudlak syndrome, lysinuric protein intolerance and chronic granulomatous disease.

Triggers: The two broad categories of triggers include those that cause immune activation and those that lead to immune deficiency. The most common infectious trigger for HLH is viral infection, especially Epstein-Barr virus (EBV).[7] Common causes of immunologic triggers include inherited syndromes, malignancy, rheumatologic disorders and HIV infection.

b. Genetics

Most of the implicated genes encode components of the machinery for perforin -dependent cytotoxicity. The presence of homozygous, compound heterozygous or digenetic mutations in a child with suspected HLH is sufficient for diagnosis. Genetic information can also be helpful in determining the likelihood of recurrence, the need for hematopoietic cell transplant and the risk of HLH in family members.

Mutations at FLH loci: PRF1/Perforin, UNC13D /Munc 13-4, STX11/Syntaxin 11 and STXBP2/Munc18-2 mutations have been the commonly identified mutations in HLH.[8]

Clinical features

Fever, hepatosplenomegaly and cytopenias are the cardinal features of HLH and should prompt the clinician to consider HLH in their list of differentials. Failure of fever to respond to first line therapy or continued deterioration despite maximal supportive care should make one suspect HLH.[9]

Initial presentation: HLH presents as a febrile illness associated with multiple organ involvement. Initial symptoms and signs of HLH can mimic common infections, fever of unknown origin, hepatitis or encephalitis. With few exceptions, the clinical features are similar regardless of presence of an underlying genetic defect. Hepatosplenomegaly, lymphadenopathy, neurologic symptoms and rash are the most common clinical features. Fever may be absent in newborns with HLH. Neonatal disseminated HSV infections may be complicated by the development of HLH.

A scoring system has been developed to generate a diagnostic score - “H score” that estimates the probability

of HLH.[10] This incorporates points for immunosuppression, fever, organomegaly, levels of triglycerides, ferritin, alanine aminotransferase and fibrinogen; degree of cytopenias and presence of hemophagocytosis on the bone marrow aspirate.

Patients with STXBP2 mutations may have hypogammaglobulinemia, severe diarrhea, bleeding and sensorineural hearing loss.

Some clinical findings are observed less frequently in affected patients from different ethnic groups. In the published series from India, the predominant presenting features included prolonged fever and hepatosplenomegaly. CNS symptoms were present in 36%. Anemia (Hb <9gm/dL) and thrombocytopenia (platelets <1, 00,000/mm^3) were present in 97% and 72% respectively. Among the biochemical markers, hyperferritinemia was present in 97%, hypofibrinogenemia and high LDH in 92%. Bone marrow examination showed hemophagocytosis in 84%. Infectious agents were identified in 42% children, with viruses accounting for two thirds of them.[11]

Neurologic findings include seizures, mental status changes (including severe changes consistent with encephalitis) and ataxia. Sometimes these findings dominate the clinical picture or develop prior to the appearance of other signs and symptoms. Patients with HLH are at risk of developing posterior reversible encephalopathy syndrome (PRES).

Respiratory abnormalities may lead to an urgent need for ventilatory support and death from acute respiratory distress syndrome (ARDS). Deteriorating respiratory function may be due to worsening of the HLH (causing an ARDS -like syndrome) or due to an infection.

Severe hypotension, renal dysfunction, skin manifestations (generalized rashes, erythroderma, edema, petechiae and purpura), bleeding and clinical features resembling Kawasaki disease have been observed.

The associated illnesses in HLH include infection, malignancy, rheumatologic and immunodeficiency syndromes. HLH is often associated with infections, especially viral such as Epstein-Barr virus, cytomegalovirus, parvovirus, herpes simplex virus, varicella-zoster virus, measles virus, human herpes virus-8, H1N1 influenza virus, parechovirus and HIV, alone or in combination. Although less common, HLH may also occur in the setting of infections due to bacteria (eg. Brucella, gram negative bacteria, tuberculosis), parasites (eg, Leishmaniasis, malaria) and fungi.

HLH is also seen in association with a number of rheumatologic diseases including systemic onset juvenile idiopathic arthritis (SoJIA), SLE and Kawasaki Disease.[12] The term macrophage activation syndrome (MAS) is used when a hemophagocytic syndrome develops in children with rheumatologic conditions. Patients with rheumatologic diseases who are treated with anti-TNF agents may get infected with Mycobacterium tuberculosis, cytomegalovirus, EBV, Histoplasma capsulatum and other bacteria and develop HLH.

Laboratory findings (Box.1)

Cytopenias: Anemia and thrombocytopenia are seen in most of the patients on presentation.

Serum ferritin: Ferritin has been described as a useful diagnostic tool in the screening for HLH. A review of ferritin levels in pediatric patients found a cut off of 10,000µg/L to be 90% sensitive and 96% specific for the diagnosis of HLH.[13] Therefore, in a critically ill child with features consistent with both sepsis and HLH, serum ferritin levels may help to direct further investigation and management of suspected cases.

Liver function and coagulation tests: Nearly all patients with HLH will have hepatitis, manifested by elevated liver enzymes (AST, ALT, GGT), LDH and bilirubin. Increased triglycerides and abnormal coagulation parameters (especially elevated D-dimer) caused by hepatic dysfunction and disseminated intravascular coagulopathy are also frequently seen. Hypertriglyceridemia may be due to severe liver involvement and may not be seen until the liver has been affected for some time.

Box 1. Essential tests in the initial workup for HLH

- Complete blood count with differential
- Coagulation studies, including PT, aPTT, fibrinogen, D-dimer
- Serum ferritin
- Liver function tests, including ALT, AST, GGT, total bilirubin, albumin and LDH
- Serum triglycerides

Other investigations which may be required depending on the clinical features include cultures of blood, bone marrow, urine, cerebrospinal fluid and other potentially infected body fluids and viral titres. Chest radiography, ECG, echocardiogram and MRI brain may be required based on the type of systemic involvement.

Bone marrow histopathology: Hemophagocytosis on bone marrow examination is variable, can be seen in different time periods and not uniformly reported. Infiltration of the bone marrow by activated macrophages is consistent with HLH. The macrophages in HLH do not have the cellular atypia associated with malignant histiocytes, and they are clearly different from the CD1a-staining Langerhans cell of Langerhans cell histiocytosis.

Immunologic profile: The following are some of the immunological investigations performed in tertiary care hematology units for evaluation of HLH (Box 2)and majority of them are not essential for initiation of therapy and not commonly available.

Box 2. Immunological investigations - HLH

- Soluble IL-2 receptor alpha (sCD25)
- Tests of NK cell function / degranulation (e.g. by flow cytometry for surface expression of the lysosomal-associated membrane protein 1 [LAMP-1, also called CD107α])
- Flow cytometry for cell surface expression of perforin and granzyme B proteins
- Flow cytometry for cell surface expression of signaling-lymphocytic-activation-molecule-associated protein (SAP) and X-linked inhibitor of apoptosis protein (XIAP) in males
- Soluble levels of the hemoglobin-haptoglobin scavenger receptor (sCD163)
- Immunoglobulin levels (e.g. IgG, IgA, IgM)
- Lymphocyte subsets (underlying immune deficiency diseases are sometimes found)

Findings consistent with HLH include elevated soluble IL-2 receptor alpha, reduced NK function or cell surface expression of CD107α, elevated sCD163 and reduced perforin, SAP or XIAP. Of all the immunologic studies, soluble IL-2 receptor alpha (sIL-2R) is found to correlate most closely with disease activity.

Genetic and HLA testing: Genetic testing (i.e. identification of an HLH gene mutation) is indicated in all patients that meet the HLH diagnostic criteria and those with a high likelihood of HLH based on the initial evaluation. Human leukocyte antigen (HLA) typing is indicated during the initial evaluation in preparation for identifying a donor for allogeneic hematopoietic cell transplantation.

Diagnostic criteria

In order to improve diagnosis of HLH, the histiocyte society published diagnostic guidelines in 1991, which were expanded in 2004.[9] Six distinct diagnostic guidelines have been used; of these, the histiocyte society's guidelines are by far the most commonly used. Five of the eight criteria are required to fulfil a clinical diagnosis of HLH, although patients with a molecular diagnosis, that is, one of the known FHL mutations, do not necessarily need to fulfil the diagnostic criteria.

Diagnostic criteria for HLH were proposed in 1991 and updated in 2004 to include NK-cell activity measured by the 51-Cr release assay, sCD25, and elevated ferritin (Box 3). These criteria, generated based on studies of FHL, are the only guidelines available for the diagnosis of acquired HLH.

The diagnostic criteria include fever, splenomegaly, cytopenias affecting at least 2 of 3 lineages in the peripheral blood; hyperferritinemia greater than 10,000 μg/L; hypertriglyceridemia and/or hypofibrinogenemia; hemophagocytosis in the bone marrow, spleen, or lymph node, low or absent NK-cell activity determined by the 51-Cr release assay and high levels of sCD25. Five of these eight criteria are required for diagnosis, although in patients with an established genetic abnormality (e.g.FHL mutations), the diagnosis can be established without meeting the 5 criteria.[14]

Box 3. Diagnostic criteria of hemophagocytic lymphohistiocytosis (HLH)[9]

Molecular diagnosis of HLH or the presence of at least 5 of 8 criteria:

1. Fever
2. Splenomegaly
3. Cytopenias (affecting at least 2 lineages in the peripheral blood)

   Hemoglobin levels <90 g/L (in infants <4 weeks old, haemoglobin <100 g/L)

   Platelets $<100 \times 10^9$/L

   Neutrophils $<1.0 \times 10^9$/L
4. Hypertriglyceridemia and/or hypofibrinogenemia:

   Fasting triglycerides ≥3.0 mmol/L (ie, >265 mg/dL)

   Fibrinogen <1.5 g/L
5. Documented hemophagocytosis in the bone marrow, spleen, or lymph nodes
6. Low or absent natural killer cell activity
7. Ferritin ≥500 μg/L
8. Soluble CD25(ie, soluble interleukin-2 receptor) ≥2,400 U/mL

Differential diagnosis and pitfalls

The main differential diagnosis of HLH is systemic inflammatory response syndrome (SIRS) due to other causes. HLH may simulate a number of common conditions that cause fever, pancytopenia, hepatic abnormalities or neurologic findings. Cytopenias, a very high ferritin level and liver function abnormalities are especially helpful in distinguishing HLH from other conditions. Absence of LFT abnormalities should prompt a thorough search for an alternative diagnosis.

The following are a few diseases which mimic HLH - Macrophage activation syndrome (MAS), infection/sepsis, liver disease/liver failure, multiple organ dysfunction syndrome, encephalitis, autoimmune lymphoproliferative syndrome (ALPS), drug reaction with eosinophilia and systemic symptoms (DRESS), Kawasaki disease, cytophagic histiocytic panniculitis, thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS) and transfusion-associated graft-versus-host disease (ta-GVHD).[15]

Treatment

Without treatment, familial HLH is often rapidly fatal and the reported mortality for secondary HLH exceeds 50%. The aim of therapy, whether for primary or secondary disease is to interrupt the amplification cascades of cytokines and suppress the hyper inflammation, with a secondary aim of killing antigen presenting cells to remove ongoing stimulus for inflammatory responses.

The HLH-94 and HLH-2004 trial protocols of the histiocyte society are based on the use of corticosteroids, etoposide and cyclosporine A. Corticosteroids are used to suppress the hypercytokinemia with dexamethasone offering the advantage of passage across the blood brain barrier thereby suppressing central nervous system inflammation. Etoposide interrupts cell division, thereby preventing the cellular proliferative response. Cyclosporine A inhibits T cell activation and cytokine production.

Both HLH-94 and HLH-2004 protocols follow an initial 8-week induction using dexamethasone and etoposide (with the earlier addition of cyclosporine from induction in HLH-2004), regardless of diagnosis of primary or secondary disease. Patients with evidence of continued or progressive central nervous system involvement after 2 weeks of systemic therapy require intrathecal therapy with methotrexate (combined with corticosteroid in the HLH-2004 protocol).Continuation therapy is recommended in all symptomatic or familial cases pending donor availability for hemopoietic stem cell transplant (HSCT). In primary cases, due to the underlying immune defect, disease is likely to recur unless the defective immune system is replaced through HSCT. Use of HSCT with both matched related and unrelated donors in the context of HLH-94 improved 3-year survival. In cases of infection-associated HLH, malignancy-associated HLH, or MAS, the immediate treatment of the underlying disease is indicated.[1,8,9]

For cases associated with acute infection, treatment of the infecting organism is recommended but may not be sufficient in isolation to resolve the condition. EBV-HLH has been managed successfully with corticosteroid or intravenous immunoglobulin but studies on Japanese adults with EBV HLH have shown survival is significantly higher, if etoposide is started within 4 weeks of diagnosis; therefore it is recommended to follow the HLH-94/2004 protocols. Isolated corticosteroid therapy and intravenous immunoglobulin therapy have both been used in MAS.

Supportive care is important during the course of treatment for HLH which includes the prophylaxis of Pneumocystis jirovecii, fungal and other opportunistic infections. Empiric broad-spectrum antibiotics or antifungal therapy should be initiated.

Recent trends in therapy of HLH included Rituximab and ATG (Antithymocyte globulin). Alemtuzumab, a monoclonal antibody to CD52, had been found to have a significant response against refractory HLH.[16-19]

Points to Remember

- ***Hemophagocytic lymphohistiocytosis (HLH) is a frequently fatal but underdiagnosed condition.***
- ***Clinical features mimic many illnesses.***
- ***Fever, lymphadenopathy, hepatosplenomegaly, rash along with bicytopenia, elevated liver enzymes and ferritin should make one suspect HLH.***
- ***Well defined criteria help in making a definitive diagnosis of HLH.***
- ***Corticosteroids, etoposide and cyclosporine A form the basis of the treatment.***
- ***Hematopoietic stem cell transplant will be needed in primary cases to correct the underlying immune defect and to prevent recurrence.***
- ***Supportive care is essential.***

References

1. Freeman HR, Ramanan AV. Review of haemophagocytic lymphohistiocytosis, Arch Dis Child 2011; 96: 688–693.
2. Filipovich A, McClain K, Grom A. Histiocytic disorders: recent insights into pathophysiology and practical guidelines. Biol Blood Marrow Transplant 2010;16: S82-S89.
3. Fall N, Barnes M, Thornton S, Luyrink L, Olson J, Ilowite NT, et al. Gene expression profiling of peripheral blood from patients with untreated new-onset systemic juvenile idiopathic arthritis reveals molecular heterogeneity that may predict macrophage activation syndrome. Arthritis Rheum 2007; 56: 3793-3804.
4. Behrens EM, Canna SW, Slade K, Rao S, Kreiger PA, Paessler M, et al. Repeated TLR9 stimulation results in macrophage activation syndrome-like disease in mice. J Clin Invest 2011; 121: 2264-2277.
5. Henter JI, Elinder G, Söder O, Hansson M, Andersson B, Andersson U. Hypercytokinemia in familial hemophagocytic lymphohistiocytosis. Blood 1991; 78: 2918-2922.
6. Osugi Y, Hara J, Tagawa S, Takai K, Hosoi G, Matsuda Y, et al. Cytokine production regulating Th1 and Th2 cytokines in hemophagocytic lymphohistiocytosis. Blood 1997; 89: 4100-41003.
7. Henter JI, Ehrnst A, Andersson J, Elinder G. Familial hemophagocytic lymphohistiocytosis and viral infections. Acta Paediatr 1993; 82: 369–372.
8. Gholam C, Grigoriadou S, Gilmour KC, Gaspar HB. Familial haemophagocytic lymphohistiocytosis: advances in the genetic basis, diagnosis and management. Clin Exp Immunol 2011; 163: 271-283.
9. Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007; 48: 124–131.
10. Fardet L, Galicier L, Lambotte O, Marzac C, Aumont C, Chahwan D, et al. Development and validation of the H Score, a score for the diagnosis of reactive hemophagocytic syndrome. Arthritis Rheum 2014; 66: 2613–2620.
11. Ramachandran B, Balasubramanian S, Abhishek N, Ravikumar KG, Ramanan AV. Profile of Hemophagocytic Lymphohistiocytosis in Children in a Tertiary Care Hospital in India, Indian Pediatr 2011; 48: 31-35.
12. Stéphan JL, Koné-Paut I, Galambrun C, Mouy R, Bader-Meunier B, Prieur AM. Reactive haemophagocytic syndrome in children with infl ammatory disorders. A retrospective study of 24 patients. Rheumatology (Oxford) 2001; 40: 1285-1292.
13. Allen CE, Yu X, Kozinetz CA, McClain KL. Highly elevated ferritin levels and the diagnosis of hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2008; 50: 1227-1235.
14. Janka GE, Schneider EM. Modern management of children with haemophagocytic lymphohistiocytosis. Br J Haematol 2004; 124: 4-14.
15. Melissa R George. Hemophagocytic lymphohistiocytosis: review of etiologies and management. J Blood Med 2014; 5: 69-86.
16. Balamuth NJ, Nichols KE, Paessler M, Teachey DT. Use of rituximab in conjunction with immunosuppressive chemotherapy as a novel therapy for Epstein Barr virus - associated hemophagocytic lymphohistiocytosis. J Pediatr Hematol Oncol 2007; 29: 569-573.
17. Mahlaoui N, Ouachee-Chardin M, de Saint Basile G, Neven B, Picard C, Blanche S, et al. Immunotherapy of familial hemophagocytic lymphohistiocytosis with antithymocyte globulins: a single- center retrospective report of 38 patients. Pediatrics 2007; 120: e622-e628
18. Horne A, Janka G, Maarten Egeler R, Gadner H, Imashuku S, Ladisch S, et al. Haematopoietic stem cell transplantation in haemophagocytic lymphohistiocytosis. Br J Haematol 2005; 129: 622-630.
19. Marsh RA, Allen CE, McClain KL, Weinstein JL, Kanter J, Skiles J, et al. Salvage therapy of refractory hemophagocytic lymphohistiocytosis with alemtuzumab. Pediatr Blood Cancer 2013; 60: 101-109.

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HEMATO ONCOLOGY

ATYPICAL PRESENTATION OF PEDIATRIC MALIGNANCIES IN OFFICE PRACTICE

***Aruna Rajendran**

Abstract: *Pediatric malignancies have a variable presentation. These may be in the form of proptosis, lytic bone lesions, testicular swelling endocrine dysfunction or simply as pyrexia of unknown origin. The diagnosis may get delayed with the use of corticosteriods and concomitant severe infection. A through knowledge of these unusual presentations and continued observation and follow up will help in arriving at the correct diagnosis. Sometimes repeating the tests like bone marrow examination on strong clinical suspicion will yield the diagnosis. Some of the variations in clinical presentation of pediatric malignancies are discussed with illustrative cases.*

Keywords: *Atypical presentation, malignancies, childhood*

Pediatric malignancies often present to pediatricians with symptoms mimicking common childhood illnesses like fever, failure to thrive, leg pain, anemia, etc. The onus is on the pediatrician to recognise these sinister diseases from the ubiquitous common benign diseases. The various atypical manifestations of common pediatric cancers are discussed by a case-based approach.

Case 1

A three-year-old child was brought with complaints of premature fall of teeth for the last 6 months. Parents have noted a depression in the right side of forehead since 1 year of age. She was otherwise normal, but had visited multiple medical care facilities for this complaint. Biopsy of the oral soft tissue lesions had revealed actinomycosis for which she was treated. Despite therapy, symptoms persisted. With the above complaints the child was re-evaluated. Skull X-ray and orthopantomogram [(OPG)

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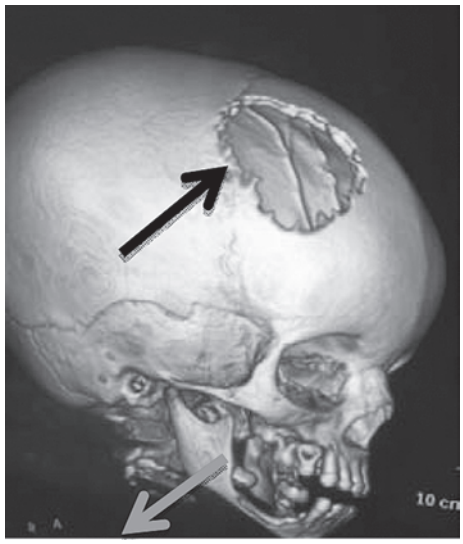

Fig.1.Osteolytic skull and mandibular lesions in a child with premature fall of teeth

(panoramic scanning dental X-ray of the upper and lower jaw)] revealed punched out lytic skull lesion and lytic lesions in both sides of the mandible respectively (Fig.1). Complete blood count and biochemical parameters were normal. Chest X-ray and ultrasound abdomen were normal. Child underwent biopsy of the mandibular lytic bone lesion which revealed the diagnosis - Langerhan cell histiocytosis (LCH). Bone marrow examination also revealed disease infiltration. She was initiated on induction therapy with steroids and vinblastine.

This child illustrates the high possibility of malignancy in children with lytic bone lesion and the importance of skeletal radiography.[1] Further, it also emphasizes the importance of sampling the appropriate area (bone rather than the soft tissue) to clinch the diagnosis.

Case 2

A four-year-old male child was brought with complaints of red coloured urine of 1 day duration. He had no history of dysuria or pain in the abdomen. Child was evaluated for possible cystitis. Child was found to have hypertension but abdominal examination was normal. Ultrasonogram followed by CT abdomen revealed 4 x 5 cm mass close to the hilum of right kidney and CT chest was normal. Child was started on hydration therapy and

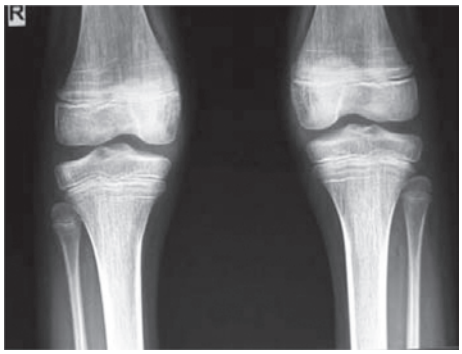

Fig.2. Leukemic lines-Radiolucent transverse metaphyseal lines

anti-hypertensives. Over next 2 days, child continued to have gross hematuria with drop in hemoglobin. In view of dropping hemoglobin child underwent right nephroureterectomy upfront which revealed stage II Wilms tumor. The child has completed 6 months of adjuvant chemotherapy and is now off treatment and doing well for more than 2 years and is on follow up.

Wilms tumor often presents as an incidentally noted mass in the abdomen. Hematuria is seen in 30% of the cases and is predominantly microscopic.[2] Occurrence of gross hematuria is rare and often causes such as cystitis and IgA nephropathy are considered initially but it is worth having an abdominal imaging to rule out surprises like renal masses and cysts.

Case 3

A three-year-old girl presented with high grade fever and severe bone pains for 2 weeks. Initially she received symptomatic therapy and had transient resolution of symptoms. As she had recurrence of the same symptoms with significant bone pain restricting her activities of daily living, she was re-evaluated (Fig.2). Investigations revealed normal blood counts and high serum calcium (13 mg/dL). There was no history suggestive vitamin D intoxication and serum 25 OH vitamin D levels were low. Her calcium progressively increased (17 mg/dL) despite hydration, forced saline diuresis and use of calcitonin. Bone marrow (BM) examination was normal. Hypercalcemia was controlled with use of pamidronate. Her previous medical records review revealed use of steroids for fever relief by the first contact physician. Therefore, despite initial normal bone marrow examination, repeat BM evaluation was done after 10 days which revealed increase in blasts (30%) and flow cytometry showed B cell acute lymphoblastic leukemia.

The above case illustrates the presentation of malignant hypercalcemia in a child with ALL and its presentation as severe bone pain.[3] It also emphasizes the avoidance of steroid (even a single dose) prior to diagnosis. This dose of steroid is detrimental as the disease often goes into transient remission which delays diagnosis and creates additional economic burden to the family and also creates drug resistant clone, decreasing effective survival.

Case 4

A nine-year-old boy was brought with high grade fever and intermittent epistaxis for 1 week. Complete blood count revealed lymphocytosis with few atypical cells and thrombocytopenia (platelet count 90,000/μL). Initial working diagnosis was acute leukemia but bone marrow evaluation revealed hypocellular reactive marrow. CT of paranasal sinuses revealed bilateral maxillary sinusitis. The child underwent functional endoscopic sinus surgery (FESS) with sinus debridement. Fungal smear and histopathology revealed broad aseptate fungus suggestive of mucormycosis. He received liposomal amphotericin for 6 weeks. Counts improved and fever subsided after a week of appropriate therapy. A month later the child presented with recurrence of fever and repeat blood counts revealed thrombocytopenia and presence of blasts in peripheral smear. Flow cytometry of bone marrow aspirate was suggestive of acute lymphoblastic leukemia - B lineage.

The above case illustrates the difficulty in establishing leukemia diagnosis in the setting of co-existent severe infection. In this scenario, a transient remission is achieved with endogenous cytokines and the disease often flares up within a period of 12 weeks.[4] These children have to be closely followed up at fortnightly intervals with a complete blood count for early identification of underlying leukemia.

Childhood cancer often presents with non-specific symptoms and signs unlike adult cancers as discussed in the case scenarios (Table I). The symptoms (fever, irritability, not gaining weight, looking pale) often mimic other common childhood illnesses. The most common childhood cancer is acute leukemia and 80%-85% of leukemia in childhood is acute lymphoblastic leukemia (ALL). ALL commonly presents with fever, lymphadenopathy, hepatosplenomegaly, pallor, bleeding and bone pains.[5] Occasionally one of the above symptoms may be the only or dominant manifestation of the disease e.g. presentation as massive hepatomegaly (mimics storage disorder, liver abscess), arthritis with near normal counts [mimics juvenile idiopathic arthritis (JIA)], generalized lymphadenopathy (mimics tuberculosis), anemia requiring blood transfusion, pyrexia of unknown origin, severe infection, isolated testicular enlargement, and acute renal failure with hypertension secondary to tumor lysis syndrome.[5]

Table I. Pediatric malignancies - Variable presentations

Symptom profile	Possible pediatric malignancies
Bilateral proptosis	Young child (< 5 years) – Neuroblastoma, LCH, AML Older child (>5 years) - Burkitt's Lymphoma, AML
Unilateral proptosis	Rhabdomyosarcoma Retinoblastoma Ewing sarcoma
Hypercalcemia	Acute leukemia Rhabdomyosarcoma
Nephrotic syndrome with lymphadenopathy	Hodgkin's lymphoma
Multiple articular swellings ± fever	Acute leukemia
Pyrexia of unknown origin	Young child - Acute leukemia, LCH, neuroblastoma Older child - Non-Hodgkin's lymphoma, Ewing's sarcoma
Hematuria ± hypertension	Wilms tumor
Hepatosplenomegaly, fever, cytopenias ± skin rash	Acute leukemias Langerhan cell histiocytosis
Bilateral cervical lymphadenopathy (Bull neck)	Acute leukemia (common in T cell& AML) Non-Hodgkin's lymphoma Hodgkin's lymphoma (unilateral > bilateral) Nasal rhabdomyosarcoma Nasaopharyngeal carcinoma
Localised lytic bone lesion	Ewings sarcoma LCH
Multiple lytic bone lesions / Bone pains	Acute leukemia Neuroblastoma Rhabdomyosarcoma
Testicular swelling	Yolk sac tumor Testicular involvement of acute leukemia
Endocrine abnormalities (Diabetes insipidus, hypothyroidism, delayed puberty)	LCH Craniopharyngioma

The awareness about these atypical presentations alerts the clinician to think upfront about acute leukemia during initial evaluation. An unaware clinician may end up treating acute leukemia with joint symptoms as JIA with drugs like steroids and methotrexate which are also the drugs used for the treatment of leukemia. This may result in transient improvement of symptoms and delay the diagnosis. Over a period of time, these partially treated children will develop drug resistant leukemic clone and will have relapse. Despite intensive treatment, this child's disease free survival will drop from 70% to a meagre 30%-40%.

Usually children with atypical presentation will have subtle clues in their complete blood count report. Presence of lymphocytosis in a febrile child should alert the clinician

to think of acute leukemia. Further the presence of bicytopenia (combination of any two cytopenias-Hb < 8g/dL, WBC count <4000/μL, platelet count <1 lakh/μL) or atypical lymphocytes in peripheral smear with or without macrocytosis warrant a bone marrow examination.

Solid tumors seen in children are brain tumors, lymphomas, neuroblastoma, Wilms tumor, germ cell tumor, retinoblastoma, hepatoblastoma and Langerhan cell histiocytosis. Lymphomas often present with localized or generalized peripheral lymphadenopathy. They can also present as (a) isolated central lymphadenopathy (mediastinal nodes, retroperitoneal nodes, iliac nodes) without palpable peripheral nodes (b) lymphadenopathy with pleural or rarely pericardial effusion and (c) focal space occupying lesions in liver and spleen. Often biopsy rather than fine needle aspiration cytology (FNAC) is required to establish a diagnosis in these children. In mediastinal disease and marked enlargement of cervical nodes (bull neck), great caution should be observed to avoid sedation, as respiratory failure is a feared complication due to airway compromise. In such a child, peripheral nodes and peripheral smear for blasts are carefully looked for and pleural fluid is examined for malignant cells rather than attempting mediastinal node biopsy. It is prudent to manage such a child in a facility with pediatric oncology services and multidisciplinary care.

Non-Hodgkin's Lymphoma especially the subtype anaplastic large cell lymphoma (ALCL) can present with fever, leukemoid reaction (polymorphonuclear leukocytosis) and lymph nodes draining pus similar to an infective illness.[6]

Neuroblastoma in an older child (>1.5 years), often presents as a locally advanced abdominal disease (stage 3) or as a metastatic disease (stage 4). Notably, neuroblastoma can have a small, non-palpable primary disease but manifest extensive metastasis. The common metastatic sites are bone, bone marrow and liver. This metastatic disease clinically presents as severe bone pain (refusal to walk, child crying on handling), pallor requiring blood transfusion, pyrexia of unknown origin or as massive hepatomegaly (especially in the new born period as a part of stage 4s neuroblastoma).[7] Such atypical manifestations should alert the clinician to search for occult primary in adrenal or retroperitoneal area. With current modern technology, nuclear scans like metaiodo benzyl guanidine (MIBG), Gallium 68 (^{68}Ga) 1, 4, 7, 10 - tetraazacyclo dodecane-1, 4, 7, 10 - tetraacetic acid DOTA - octreotate (^{68}Ga-DOTATATE) PET/CT scans can be utilized to look for neuro-endocrine tumors.

Germ cell tumors commonly manifest as testicular swelling, abdominal mass or mediastinal mass (with or without respiratory distress), often solid or cystic in consistency. It is worth to do serum alpha fetoprotein (AFP) and beta human chorionic gonadotrophin (β HCG) in all cases with abdominal and mediastinal masses as there are reports of teratomas presenting even as renal masses.[8] Pelvic germ cell tumors can compress the ureters and present as obstructive uropathy with oliguria and renal failure.[9]

Langerhan cell histicytosis (LCH) during infancy presents as multi-systemic disease manifesting as fever, hepato-splenomegaly, cytopenias (anemia, thrombocytopenia), with or without skin rash and ear discharge. A simple plain X-ray of skull showing punched out osteolytic lesions or skin biopsy in a child with rash which will help us establish a diagnosis and rule out a wide range of differential diagnosis such as TORCH infections, familial hemophagocytic lymphohisticytosis (HLH), disseminated tuberculosis and primary immunodeficiency.

LCH can also present as central diabetes insipidus by causing hypophyseal masses.[10] Another tumor causing significant endocrinological morbidity is craniopharyngioma which also manifests with combination of any of the following symptoms: headache, vomiting, visual field defects, morbid obesity, failure to gain weight, polyuria and polydipsia.[11,12]

Conclusion

In the current era, pediatric malignancies have high cure rate, especially if identified early and managed in tertiary care centre capable of providing multidisciplinary care. The role of pediatricians in oncology care begins from the time of diagnosis and extends during treatment and follow up. Knowledge about such atypical presentations of pediatric cancers will help the first contact physicians to make the right diagnosis and guide the parents appropriately.

Points to Rembember

- ***Pediatric malignancies may present with clinical features of other common childhood problems, such as infection, endocrine problems, rheumatologic disorders etc.***
- ***The diagnosis may also get delayed due to iatrogenic factors such as even a single dose of steroids.***
- ***In clincally suspected cases, continuous reexamination and repeating the tests like bone marrow examination will help in the diagnosis.***

- ***Appropriate tissue should be sampled for accurate diagnosis.***
- ***Knowledge about the atypical presentation will help the pediatrician to suspect malignancy in the appropriate clinical circumstances.***

References

1. Golai S, Nimbeni B, Patil SD, Kakanur M, Paul S. Langerhans histiocytosis in a child -diagnosed by oral manifestations. J Clin Diagn Res 2015;9:9-11.
2. Kalapurakal JA, Dome JS, Perlman EJ, Malogolowkin M, Haase GM, Grundy P, et al. Management of Wilms' tumour: current practice and future goals. Lancet Oncol 2004; 5: 37–46.
3. Sargent JT, Smith OP. Haematological emergencies managing hypercalcaemia in adults and children with haematological disorders. Br J Haematol 2010; 149:465-477.
4. Rajendran A, Trehan A, Ahluwalia J, Marwaha RK. Severe Systemic Infection Masking Underlying Childhood Leukemia. Indian J Hematol Blood Transfus 2013; 29:167–170.
5. Margolin JF, Rabin KR, Steuber CP, Poplack DG. Acute lymphoblastic leukemia. In: Pizzo PA, Poplack DG, eds. Principles and Practice of Pediatric Oncology. 6th edn. Philadelphia Pa: Lippincott Williams & Wilkins, 2011.
6. El-Osta HE, Salyers WJ Jr, Palko W, Hagan ME, El-Haddad B, Schulz TK. Anaplastic large-cell lymphoma with leukemoid reaction. J Clin Oncol 2008;26: 4356-4358.
7. Lew T, Chauhan A, Vasquez R, Warrier R. Massive Hepatomegaly with Respiratory Distress in a Newborn. Clin Pediatr 2015; 54:907-909.
8. Lin S, Li X, Sun C, Feng S, Peng Z, Huang S, et al. CT findings of intrarenal yolk sac tumor with tumor thrombus extending into the inferior vena cava: a case report. Korean J Radiol 2014;15:641-645.
9. Binder Z, Iwata K, Mojica M, Ginsburg HB, Henning J, Strubel N, et al. Acute Urinary Retention Caused by an Ovarian Teratoma-A Unique Pediatric Presentation and Review. J Emerg Med 2015;49: e139-142.
10. Horn EM, Coons SW, Spetzler RF, Rekate HL. Isolated Langerhans cell histiocytosis of the infundibulum presenting with fulminant diabetes insipidus. Childs Nerv Syst 2006;22:542-544.
11. Müller HL. Craniopharyngioma. Endocr Rev 2014; 35:513–543.
12. Keates-Baleeiro J, Rincon M. Morbid Obesity as Early Manifestation of Occult Hypothalamic-Pituitary LCH with Delay in Treatment. Case Rep Oncol Med 2015;915716. doi: 10.1155/2015/915716. Epub 2015 Nov 30.

NEWS AND NOTES

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HEMATO ONCOLOGY

MANAGEMENT OF COMMON PROBLEMS DURING LEUKEMIA TREATMENT

***Anupama Borker**
****Pooja Balasubramanian**

Abstract: *Leukemia is the most common and curable of childhood cancers. The treatment of acute leukemia in children is intense and prolonged. Chemotherapy-induced myelosuppression leads to anemia, neutropenia and thrombocytopenia. The prompt treatment of bacterial and fungal infections, rational use of blood components and the availability of potent anti-emetics and analgesics have made leukemia treatment safer. Malnutrition, depression and physical disability during leukemia therapy can be effectively treated with nutritional rehabilitation, physiotherapy and psychosocial support respectively.*

Keywords: *Leukemia, Chemotherapy, Neutropenia, Anemia.*

The treatment of acute leukemia in children is intense and prolonged. The improved cure rates of children with leukemia over the last few decades is largely due to the improvement in supportive care which includes better antibiotics and antifungal drugs, blood component therapy, better antiemetic drugs and potent analgesics.

Neutropenic infections

In acute leukemia, neutropenia occurs because of the disease and its treatment. The chemotherapy used for treating acute leukemia is myelosuppressive leading to variable periods of neutropenia. The intensity of regimens used and patient and disease related factors affect depth and duration of the neutropenia which directly affects the incidence of neutropenic fever.

Febrile neutropenia (FN) is defined as the presence of fever with a single temperature recording of 101^{o}F or sustained temperature of 100.4^{o}F for at least one hour, in a patient with neutropenia [absolute neutrophil count (ANC) less than 500/cmm] or likely to fall below 500/cmm in the next 48 hours.[1] Febrile neutropenia is an emergency as bacteremia can advance rapidly in a neutropenic host to cause septic shock and death within a few hours. Evaluation of patients presenting with FN should be done promptly with the aim to administer the first dose of antibiotic within 30 minutes of admission. A good history, detailed physical examination and details of previous episodes of FN should be obtained.

Neutropenic patients are unable to mount a full inflammatory response. Subtle signs like mild erythema and pain should be attributed to infection. The oropharynx, respiratory tract, gastrointestinal tract, perianal area, sites of central line and recent invasive procedures are the commonest sites of infection. After obtaining blood cultures from peripheral and central venous lines, broad spectrum antibiotics should be started at the earliest.

The infectious disease society of America guidelines for empiric antibiotic use in FN recommend starting monotherapy with piperacillin/tazobactam, carbapenems, ceftazidime or cefipime depending on the prevalent patterns of organisms and antimicrobial sensitivity in a given center. The indications for adding vancomycin on admission include suspected central line infections, presence of cellulitis, typhlitis or severe enterocolitis or hypotension suggestive of sepsis.[2] Patients with known colonization with penicillin or cephalosporin resistant pneumococci or MRSA also merit vancomycin. Subsequent antibiotic changes should be dictated by culture reports. The indication for starting antifungal therapy is persistent fever after 5 days of antibiotics, especially if neutropenia is expected to last another 4 days, previous documented fungal infection or serological or imaging evidence of fungal infection. Febrile neutropenia is often accompanied by anemia and thrombocytopenia. Maintaining a hemoglobin above 8 gm/dL and a platelet count above 20,000/cmm is advisable during FN.

Fungal infections

Common fungal infections during leukemia treatment include candidiasis and aspergillosis; mucormycosis is less common. Invasive fungal infections (IFI) can involve single

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organ or it can be disseminated. Invasive candidiasis (IC) most commonly affects the blood stream whereas invasive aspergillosis (IA) is more common in the lungs and sinuses.[3] Liver, spleen, kidneys and brain can also be affected. Thrush and oropharyngeal candidiasis are common during periods of steroid therapy in the treatment of leukemia. Patients at high risk for IFI include those on steroid therapy during induction and reinduction cycles, those with prolonged and profound neutropenia, i.e., ANC less than 100/cmm for 3 weeks or less than 500/cmm for 5 weeks and those colonized with candida.[4]

In patients with suspected fungal infections, positive blood or urine culture for candida species can prove the infection. For aspergillosis the yield of blood culture is extremely low and serological tests with Galactomannan or 1,3-beta-D glucan are more reliable.[5] Galactomannan which has high sensitivity and specificity for IA, also has value in monitoring response to therapy after diagnosis.

Imaging studies play a crucial role in diagnosing IFI. Chronic disseminated candidiasis is characterized by small peripheral target-like lesions (bull's eye lesions) in the liver and spleen. Findings such as a halo sign, air crescent sign, or cavity within an area of consolidation on CT scan of the chest is highly suggestive of angio-invasive fungal pneumonia.[6] Many patients with leukemia receive anti-fungal prophylaxis with fluconazole, voriconazole or posaconazole during high risk periods in their therapy.

Empiric antifungal therapy is started in leukemia patients after 5 days of fever during neutropenic episodes or with radiological or serological evidence of fungal infection. The primary treatment for IC in neutropenic cancer patients is either liposomal amphotericin B or an echinocandin such as caspofungin or micafungin. Treatment is prolonged and is to be continued for 2 weeks after neutropenia and symptoms resolve and all relevant cultures are sterile. The primary treatment for IA is voriconazole, though liposomal amphotericin B may be used as an alternative. Children need higher doses of voriconazole and therapeutic drug monitoring is recommended especially in those with proven aspergillosis. Surgical resection is needed for lesions in close proximity to the pericardium or great vessels or those eroding into the pleura and ribs, or causing hemoptysis.

Blood component support

Myelosuppression is integral to leukemia therapy. Apart from neutropenia, anemia and thrombocytopenia can vary in severity depending on treatment intensity and patient factors.

During the intensive phases of leukemia therapy, anemia occurs due to multiple causes including the myelosuppressive effect of chemotherapy, underlying poor iron stores, decreased nutrient intake, poor absorption due to mucositis and increased losses due to bleeding (menorrhagia, occult blood in stools and repeated venipunctures).

The treatment of anemia is individualized. In a stable afebrile patient without any active infection, transfusion with packed red cells is needed only if hemoglobin drops below 7 or 8 gm/dL.[7] Packed red cell transfusions may be needed at higher hemoglobin levels in patients with pneumonia, sepsis or active bleeding.[5]

Ideally leukemia patients should receive leucoreduced and irradiated packed RBCs. Leucoreduction is done using WBC filters either at source (in the blood bank just after collection from the donor) or at the bedside. It helps to reduce the incidence of alloimmunisation in multiply transfused patient. Irradiating the packed RBC bag at a dose of 25 Gy destroys lymphocytes and reduces the risk of transfusion associated graft versus host disease (TAGVHD) in this immunocompromised population.[8] Directed donations from first degree relatives should be strongly discouraged in leukemia patients to prevent TAGVHD.

Most chemotherapy cycles lead to transient thrombocytopenia in leukemia patients. The aim of platelet transfusion is to prevent bleeding and not to correct platelet counts. In a stable afebrile patient without any clinical bleeding, transfusion is indicated if platelet count is less than 10,000/cmm. In a patient with febrile neutropenia, platelet transfusion is indicated to maintain platelet count above 20,000/cmm and above 40,000 to 50,000/cmm in patients with hyperleucocytosis at diagnosis, pneumonia, severe mucositis, active bleeding, concurrent coagulation abnormality or prior to any invasive procedure like bronchoscopy.

Single donor platelets (SDP) obtained by apheresis of donors is equivalent to approximately 6 units of random donor platelets that are obtained from centrifugation of donated whole blood. Leukemia patients who are likely to need multiple transfusions should receive SDP to limit donor exposure and subsequent refractoriness due to alloimmunisation. Just as in packed RBC transfusions, leucoreduction and irradiation of platelets helps in reducing the risk of alloimmunisation and TAGVHD respectively.

Thrombosis

Thromboembolic episodes are among the serious and

more frequent complications of ALL and its treatment. A combination of disease, host and treatment related risk factors play a role in the causation of thrombotic events. A prothrombotic state may be produced during malignancy due to direct synthesis of procoagulant molecules and inflammatory cytokines by cancer cells or their interaction with vascular endothelial cells. The majority of episodes occur in the induction phase of chemotherapy when drugs such as asparaginase and prednisolone are used which cause alteration in the levels of circulating coagulant and anticoagulant factors.

Central venous line (CVL) related thromboses are common in children with ALL.[9] Once diagnosed, immediate removal of CVL and prompt anticoagulation can result in quick resolution and early recovery. Three months of anticoagulation is recommended with twice daily low molecular weight heparin (LMWH) to avoid the problem of drug interaction with use of warfarin. Once daily prophylactic LMWH should be started on re-exposure to asparaginase and for 48 hours after the last dose (or 2 weeks after pegylated asparaginase).[10] Anticoagulation in patients with severe thrombocytopenia requires careful management to avoid serious bleeding complications. Platelet transfusions to maintain a platelet count of at least 30-50 X 10^9/L or reduction or cessation of heparin during periods of thrombocytopenia may be done.

Mucositis

Mucositis is the painful inflammation and ulceration of mucous membranes lining the digestive tract, occurring as an adverse effect of chemotherapy. It may range from mild inflammation to deep ulcerations involving any part from the mouth to the anus. The World Health Organization (WHO) oral toxicity scale grades the severity from 0 (no oral mucositis) to 4 (patient requiring total parenteral nutrition) based on anatomical, symptomatic and functional components of mucositis. Symptoms of mucositis include pain, bleeding, dysphagia, infections and reduced oral intake. This in turn leads to longer periods of hospitalization, significant increase in costs and affects timely administration of treatment.

Management of mucositis depends on its severity and expected duration. Good oral hygiene, regular dental care and patient education are important components of the management. Pain related to mastication and swallowing is relieved by the cessation of oral feeds and instituting intravenous fluids and parenteral nutrition therapy. Pain relief achieved with analgesic therapy also helps to resume oral feeding early and decreases the duration of hospital stay. The mainstay of analgesic therapy is parenteral administration of opioids, as topical analgesics and anesthetics are of limited use. Control of mild to moderate pain can be achieved with narcotic analgesics like tramadol and codiene,[11] while intravenous morphine is recommended for more severe pain. Palifermin, a human recombinant keratinocyte growth factor (KGF)[12] and two human fibroblast growth factors (repifermin, velafermin)[13] are newly discovered targeted therapies for prevention of mucositis.

Nutrition

Malnutrition is common among patients with leukemia. Adequate calorie intake may be affected by the disease itself and the side effects of therapy (nausea, vomiting, anorexia, stomatitis, dysphagia, changes in taste). Along with weight loss, wasting and stunting, delayed wound healing, increased infectious complications, decreased immune competence, reduced respiratory and other muscle strength and increased length of hospital stay have been documented due to manutrition.[14]

It is also found to be associated with intolerance to chemotherapy and increased mortality rates. The clinical assessment and the child's nutrient requirements help to determine the method of nutrition support. Modifications to the oral diet with calorie supplementation help to achieve weight gain during treatment. This can be accomplished by combining regular home cooked food with commercial supplemental drinks, bars or calorie additives. Tube feedings should be considered as a means of preserving or obtaining optimal nutritional status if nutrient needs are not met orally. Tube feeding helps to improve weight gain and nutritional status without the cost and risk associated with parenteral nutrition.

Parenteral nutrition should be resorted to in patients with severe gut abnormalities where enteral nutrition cannot be given for prolonged periods. Total parenteral nutrition is associated with significant risk factors such as infections, hepatotoxicity and metabolic abnormalities.

Psychosocial problems

The social life and the psychological status of children are significantly affected following the diagnosis of leukemia. Psychosocial problems arise due to the disease and the intensive nature of the treatment including chemotherapy and cranial radiotherapy. These include depression, sleep disturbances, chronic fatigue syndrome, attention and concentration deficits, impaired auditory and visual short-term memory, reduced speed of processing, lower scores in global and verbal IQ's and finally, learning disabilities.

Frequent assessment by child psychologists and counsellors helps in early detection of these problems. School plays a vital role in the psychosocial development of children and must be resumed as soon as the intensive part of the treatment is completed. Counselling the school teachers regarding the child's disease helps in the rehabilitation of the child when they resume school.

Points to Remember

- ***Febrile neutropenia is an oncological emergency requiring timely action with antibacterial and antifungal therapy to avoid mortality.***
- ***Transfusion with leucodepleted and irradiated blood products helps to prevent alloimmunisation and transfusion associated graft versus host disease in children with leukemia.***
- ***Aggressive nutritional rehabilitation must be initiated from the time of diagnosis to withstand chemotherapy and reduce morbidity and mortality.***
- ***Along with physical problems, emotional and social needs of the child must also be addressed with establishment of a normal routine with age appropriate activities.***

References

1. Hughes WT, Armstrong D, Bodey GP, Bow EJ, Brown AE, Calandra T et al. 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clin Infect Dis 2002;34: 730-751.
2. Freifeld AG, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA, et al. Clinical Practice Guideline for the Use of Antimicrobial Agents in Neutropenic Patients with Cancer: 2010 Update by the Infectious Diseases Society of America. Clinical Infect Dis 2011;52: e56–e93.
3. Hammond SP, Marty FM, Bryar JM, DeAngelo DJ, Baden LR. Invasive fungal disease in patients treated for newly diagnosed acute leukemia. Am J Hematol 2010; 85: 695–699.
4. Prentice HG, Kibbler CC, Prentice AG. Towards a targeted, risk-based, antifungal strategy in neutropenic patients. Br J Haematol 2000; 110: 273–284.
5. Pazos C, Ponton J, Del Palacio A. Contribution of (1→3)-beta-D-glucan chromogenic assay to diagnosis and therapeutic monitoring of invasive aspergillosis in neutropenic adult patients: A comparison with serial screening for circulating galactomannan. J Clin Microbiol 2005; 43: 299–305.
6. Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F et al. Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer; Mycoses Study Group of the National Institute of Allergy and Infectious Diseases. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: An international consensus. Clin Infect Dis 2002; 34: 7-14.
7. Barnard DR, Rogers ZR. Blood component therapy. In: Altman AJ, ed. Supportive care of children with cancer. 3rd Edn, Current therapy and guidelines from the Children's Oncology Group. Baltimore and London: The Johns Hopkins University Press, 2004;pp39-57.
8. Luban NL, Drothler D, Moroff G, Quinones R. Irradiation of platelet components: inhibition of lymphocyte proliferation assessed by limiting-dilution analysis. Transfusion 2000;40:348-352.
9. Wermes C, von Depka Prondzinski M, Lichtinghagen R, Barthels M, Welte K, Sykora KW. Clinical relevance of genetic risk factors for thrombosis in pediatric oncology patients with central venous catheters. Eur J Pediatr 1999; 158: S143–146.
10. Payne JH, Vora AJ. Thrombosis and acute lymphoblastic leukemia. Br J Haematol 2007;138: 430-445.
11. Niscola P, Scaramucci L, Romani C, Giovannini M, Maurillo L, del Poeta G, et al. Opioids in pain management of blood-related malignancies. Ann Hematol 2006;85: 489-501.
12. Spielberger R, Stiff P, Bensinger W, Gentile T, Weisdorf D, Kewalramani T, et al. Palifermin for oral mucositis after intensive therapy for hematologic cancers. N Engl J Med 2004;351: 2590-2598.
13. Braun S, Hanselmann C, Gassmann MG, auf dem Keller U, Born-Berclaz C, Chan K et al. Nrf2 transcription factor, a novel target of keratinocyte growth factor action, which regulates gene expression and inflammation in the healing skin wound. Mol Cell Biol 2002;22: 5492-5505.
14. Chima CS, Barco K, Dewitt ML, Maeda M, Teran JC, Mullen KD. Relationship of nutritional status to length of stay, hospital costs, and discharge status of patients hospitalized in the medicine service. J Am Diet Assoc 1997;97(9):975-978.

HEMATO ONCOLOGY

RECENT ADVANCES IN THE MANAGEMENT OF PEDIATRIC SOLID TUMORS

***Prakash Agarwal**

Abstract: *Solid tumors make up about 30% of all pediatric cancers. The most common types of solid tumors in children include brain tumors, neuroblastoma, rhabdomyosarcoma, Wilms' tumor and osteosarcoma. In the last decade substantial progress has been made in the treatment of pediatric solid tumors. Better understanding of the natural history of the various tumors, improved histologic classifications, new techniques to define extent of disease accurately, effective chemotherapy and improved radiation, surgical and supportive therapies have contributed to improved survival. This article reviews some of the common childhood tumors, emphasizing on current management and future directions.*

Keywords: *Pediatric solid tumors, Recent advances.*

Survival rate for childhood cancer has improved dramatically over the last 50 years. Although we continue to make progress, there is still scope for improvement in developing countries.[1]

The multidisciplinary approach to cancer treatment involving pediatric surgeons, radiation therapists and pediatric oncologists has improved the treatment and outcome of solid tumors in children.[2] The successful use of a combination of chemotherapeutic agents has led to the widespread use of combination chemotherapy to treat virtually all types of pediatric cancers. Since the late 1980s, neuroblastoma has been the paradigm for the use of therapies of variable intensity, depending on risk stratification determined by clinical and biologic variables, including molecular markers.[3]

Other advances in pediatric oncology have included the development of interdisciplinary, national cooperative clinical research groups to critically evaluate new therapies, the efficacy of dose-intensive chemotherapy programs in improving the outcome of advanced-stage solid tumors, and the supportive care necessary to make the latter approach possible. The development and application of these principles and advances have led to substantially increased survival rates for children with cancer and profound improvements in their quality of life.[3]

Advances in molecular genetic research have led to an increased understanding of the genetic events in the pathogenesis and progression of human malignancies, including those of childhood.[3]

Incidence of solid tumors in children: The annual incidence of cancer in children under 15 years of age is usually between 100 and 160 per million. There is a risk of 1 in 650 to 1 in 400 that a child will be affected during the first 15 years following birth.[1] Leukemia is the most common form of cancer in children, and brain tumor, the most common solid tumor of childhood (Table I). Lymphomas are the next most common malignancy in children, followed by neuroblastoma, soft tissue sarcomas, Wilms' tumor, germ cell tumors, osteosarcoma and retinoblastoma.[3]

Recent advances in the management of pediatric solid tumors encompass diagnostic and therapeutic modalities.

Table I. Type of cancer in childhood

Types of cancer	Percentage of total
Leukemia	30
Brain tumors	22
Lymphoma	15
Neuroblastoma	8
Sarcoma	7
Wilms' tumor	6
Germ cell tumors	5
Osteosarcoma	4
Retinoblastoma	2
Liver tumors	1

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Diagnostics comprise of molecular diagnostics, imaging techniques and newer techniques in histopathology. The newer treatment modalities are in the form of targeted chemotherapy.

Molecular diagnostics

Information about the human genome has led not only to an improved understanding of the molecular genetic basis of tumorigenesis but also to the development of a new discipline: the translation of these molecular events into diagnostic assays. The field of molecular diagnostics has developed from the need to identify abnormalities of gene or chromosome structure in patient tissues and as a means of supporting standard histopathologic and immunohistochemical diagnostic methods. In most instances, the result of genetic testing confirms light microscopic and immunohistochemistry-based diagnosis.

In some instances molecular analysis is required to make a definitive diagnosis (e.g. primitive small round cell tumor, poorly differentiated synovial sarcoma and lipoblastic tumor).

The molecular genetic methods most commonly used to analyze tumor material include, direct metaphase cytogenetics or karyotyping, fluorescent in situ hybridization (FISH) and reverse transcriptase polymerase chain reaction (RT-PCR).

Additional methods such as comparative genomic hybridization, loss of heterozygosity analysis and complementary DNA (cDNA) microarray analysis may eventually become part of the routine diagnostic repertoire but are currently used as research tools at referral centers and academic institutions.

The value of molecular genetic analysis of patient tissue is not limited to aiding histopathologic diagnosis. Many of the most important markers provide prognostic information as well. For example, MYCN amplification in neuroblastomas,[4] is strongly associated with biologically aggressive behavior. Amplification of this gene can be detected by routine metaphase cytogenetics or by FISH and current neuroblastoma protocols include the presence or absence of MYCN amplification in their stratification schema.

New technologies are emerging that permit accurate, high-throughput analysis or 'profiling' of tumor tissue: gene expression can be analyzed by using RNA microarrays and proteins by using proteomics. These approaches identify a unique 'fingerprint' of a given tumor that can provide diagnostic or prognostic information. Proteomic analysis can also identify unique proteins in patients' serum or urine; such a profile can be used for early tumor detection, to distinguish risk categories and to monitor for recurrence.

Once the quality and consistency of sample material can be refined and data management and statistical analyses validated and standardized, gene profiling microarrays will probably be used routinely to analyze pediatric malignancies.

Imaging techniques

CT scan/MRI

Although CT is well established, it utilizes ionizing radiation and therefore modern imaging of most tumors in the pediatric population should preferentially be performed by MRI where this is feasible. Furthermore, MRI has advanced so rapidly in recent years that it is now considered the superior modality (outside the lungs) irrespective of the radiation issues surrounding CT. However, CT is generally more readily available, cheaper, and easier for the patient than MRI and new scanners are now so fast that patients who previously required a general anesthetic for CT may now be able to have the scan performed under sedation or even unsedated. CT provides excellent images of lung parenchyma, mediastinum, head and neck, abdomen and pelvis. Intravenous injection of contrast medium is essential to delineate mediastinal masses, hepatic tumors, renal masses and head and neck tumors and can provide superior vessel details by CT angiography compared to conventional angiography (Fig.1). Spiral CT rotates the X-ray beam and the diametrically opposing detectors around the patient. Modern scanners have multiple detectors (typically 64 or 128) with the capability of very thin slice thicknesses (as low as 0.625 mm) allowing very rapid scanning of large body areas that can be completed in 5–10 sec which, with extensive image post processing and manipulation, can finally produce reconstructed planar and 3D images.

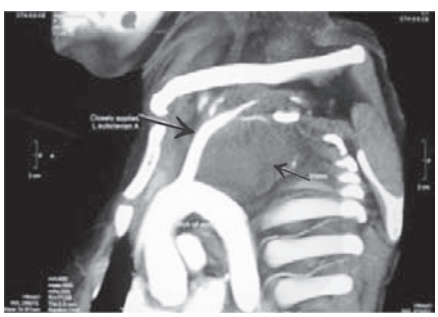

Fig.1. CT scan with contrast showing the relationship of tumor to great vessels of a superior mediastinal tumor

Magnetic resonance imaging (MRI) provides exquisite anatomical detail of many pediatric tumors. It has advantages over CT and US scanning including greater inherent tissue contrast, multiplanar imaging and non-invasive angiography and avoids exposure to ionizing radiation.

New super-fast sequences and improving scanner design (i.e., 'open-MR') is transforming the feasibility of MRI in the pediatric age group. MRI should be considered the imaging modality of choice for tumors of the musculoskeletal system, central nervous system, including the spine, head and neck tumors, as well as the abdomen, pelvis and mediastinum. Modern sequences and the use of contrast agents can also give information about the vascularity and enhancement characteristics of tumors. This can also be of benefit in the assessment of vascular malformations. Magnetic resonance angiography (MRA) is particularly useful in evaluating tumor proximity or involvement of major vessels. Whole-body MR may also compete with positron emission tomography (PET) imaging to stage abdominal tumors. Specific advantages of MR include determination of resectability of hepatic tumors using combined MR and MRA; staging of neuroblastoma in the bone marrow, lymph nodes, liver and spinal canal; response of bilateral Wilms' tumor and nephroblastomatosis; and detection of pelvic tumors with sagittal sections, and peritoneal tumors with contrast enhancement.[5]

Fast spin-echo short inversion time inversion-recovery (STIR) whole-body (MR) imaging is an evolving technique that allows imaging of the entire body over a reasonable period of time. Its wide availability and lack of radiation exposure makes this method appealing in children. Bone marrow lesions, including marrow infiltration from lymphoma, metastases and tumor-related edema, are observed with high signal intensity and are more easily detected on STIR images than with scintigraphy. Focal parenchymal lesions can be distinguished by their slightly different signal intensity, but pathologic lymph nodes cannot at present be differentiated from normal nodes on the basis of signal intensity. The STIR technique is highly sensitive for detection of pathologic lesions, but it is not specific for malignancy; thus, the method cannot be used to differentiate benign conditions from malignant neoplastic lesions with certainty at present.[6]

Nuclear imaging

Nuclear scintigraphy is useful in diagnosis, staging, and assessment of tumor response and evaluation of treatment in various pediatric tumors. The technological aspects of radioisotope scanning are particularly important when imaging children.

Image magnification and single photon emission computed tomography (SPECT) are essential to state-of-the-art pediatric nuclear medicine. Multiple head detector gamma camera systems are available and have the advantages of increased resolution and sensitivity and decreased time of examination in a child.

Nuclear imaging techniques such as bone scans, meta-iodo-benzyl guanidine (MIBG) scans, and Indium111-diethylenetriaminepentaacetic acid (DTPA) octreotide scans have greatly increased the sensitivity and specificity of both diagnostic and follow-up protocols for pediatric solid tumors. Molecular targets that are specific for certain pediatric tumors are now being developed. Targets include cell membrane receptors targeted by specific ligands (such as octreotide), subcellular organelles targeted by false transmitters (such as MIBG) and cellular proteins targeted by antibodies.[7]

Positron emission tomography

Positron emission tomography (PET) involves the acquisition of physiologic images based on the detection of radiation from the emission of positrons. Positrons are emitted from a radioactive substance fluorodeoxyglucose (FDG) administered to the patient. Different color intensities on a PET image represent different levels of tissue glucose metabolism. Healthy tissue accumulates some of the tagged glucose, which shows up on the PET images. However, cancers, which use more glucose than normal tissue, accumulate more of the substance and appear brighter than normal tissue on the PET images (Fig. 2).

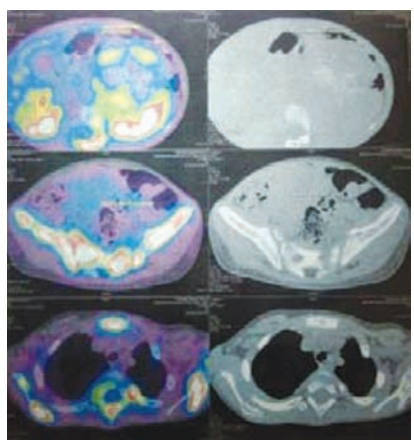

Fig.2. PET scan showing the various color coding to differentiate between normal and cancer cells depending on the uptake of tagged glucose

PET scans are useful both in the detection of cancer and in examining the effects of cancer treatment by characterizing biochemical changes in the cancer. PET may be combined with CT to exactly localize areas of abnormal tissue, both for biopsy and to plan surgery. PET scans are now being used more widely in the pediatric population although usually only in specialized cancer centers.

Biopsy

The importance of biopsy techniques has increased as the use of preoperative chemotherapy has become common for many childhood cancers. In the past, definitive diagnosis was made at the time of surgical resection of the primary tumor. Currently, many children undergo percutaneous or open incisional biopsy rather than initial resection. Moreover, with a better understanding of the molecular changes associated with these malignancies, definitive diagnosis and accurate staging can be accomplished with smaller specimens. This should lead to less morbidity associated with the diagnosis of solid malignancies in children.

There has been a progression towards less invasive techniques to obtain a diagnosis, from complete surgical extirpation to incisional biopsy to percutaneous needle biopsy and minimal-access surgery. This change in practice has been driven not only by the evolution of surgical technique but also by an improved understanding of the molecular markers used for both diagnosis and risk stratification in pediatric solid malignancies.

The types of specimens which might be submitted to the pathologist are cytology specimen, needle biopsy, incisional biopsy, excisional biopsy and resected specimen.

Fine needle aspiration cytology (FNAC) is used less frequently in children. The recent application of molecular techniques and electron microscopy to supplement light microscopy has increased the histio-type specificity of FNAC and may lead to its increased application in pediatric solid malignancy.[8]

Percutaneous needle biopsies may be performed by palpation in the extremities and other superficial locations, such as lymph nodes. Deeper biopsies require either ultrasonography or computed tomography (CT) guidance.

Minimal access surgery: The widespread use of minimal-access surgery, including laparoscopy and thoracoscopy, has had a significant impact on general pediatric surgery over the last 25years. The application of both laparoscopy and thoracoscopy has now grown to include the initial diagnosis of childhood malignancies and the assessment of refractory and metastatic disease. Diagnostic laparoscopy and biopsy have been used in several settings in the management of children with solid malignancies. Biopsies obtained using laparoscopic techniques have a high rate of success in yielding diagnostic tissue. Laparoscopy allows the surgeon to obtain larger tissue samples than may be obtained with core needle biopsy.[9] This is particularly relevant if larger samples are required for biologic studies. In the initial diagnosis, laparoscopy aids in identifying the site of origin of large abdominal masses. Laparoscopy is superior to CT in assessing intraperitoneal neoplasms and for the evaluation of ascites. Thoracoscopy is frequently used to evaluate metastases either at the time of initial diagnosis or after follow-up imaging. Mediastinal lesions can also be biopsied or resected using thoracoscopy.[10]

Immunohistochemistry

The introduction of immunohistochemistry to the armamentarium of diagnostic histopathology has revolutionized the subject and has significantly contributed to the management of pediatric neoplasia. In recent years there has been a massive expansion in the use of antibodies in tissue diagnosis. Immunohistochemistry is based on the technique that a particular component of a tissue, acting as an antigen, can be identified by a specific antibody carrying a label which can be rendered visible (Fig.3).

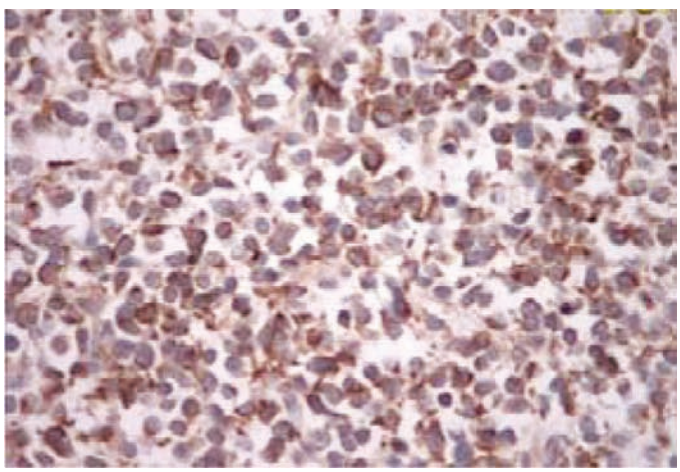

Fig.3. Immunohistochemistry of rhabdomyosarcoma showing positivity for desmin

Two types of antibodies are used. The first are polyclonal and tend to be less specific and sensitive while the second, which are now more commonly used, are monoclonal antibodies which allow for the use of very sensitive and highly specific detection techniques. Example panels of some antibodies commonly used in the diagnosis of pediatric tumors are provided in Table II.

Table II. Antibodies useful in pediatric tumor diagnosis

Antibody	Antisera/ Cell lineage marked	Tumor
CD45 Leukocyte common antigen	Leukocytes	Lymphomas
CD20 (L26)	B lymphocytes	Lymphomas
CD45RO(UCHL-1)	T lymphocytes	Lymphomas
CD30 (Ber H-2)	Activated lymphocytes/macrophages/ Reed-Sternberg cells	Hodgkin's disease/Anaplastic large cell lymphoma
CD15 (LeuM1)	Reed-Sternberg cells	Hodgkin's disease
CD68 (KP1)	Macrophages	Histiocytic neoplasms
Kappa/Lambda	Ig light chains	Lymphoid clonal proliferation
Neuron-specific enolase (NSE)	Neuroectoderm	Neuroblastoma
S100	Glial/Schwann cells/others	Neurofibroma, etc., Langerhans cells
β2-microglobulin	β2-microglobulin	PNET
Synaptophysin	Neuroectoderm/neuroendocrine	Ewing's/PNET
MIC-2 (CD99)	MIC-2 gene product (glycoprotein P30/32)	Ewing's/PNET
Vimentin	Intermediate filaments/mesenchyme	Ewing's/soft tissue sarcoma
Actin (common, smooth muscle, sarcomeric)	Muscle filaments	Rhabdomyosarcoma
Desmin	Muscle (smooth/striated)	Rhabdomyosarcoma
Myoglobin	Striated muscle	Rhabdomyosarcoma
Myo D-1	Skeletal muscle	Rhabdomyosarcoma
Cytokeratins (AE1-AE3, CAM 5.2,etc.)	Epithelial	Synovial sarcoma
CD1a	Langerhans cells	Langerhans cell histiocytosis

Flow cytometry

In the case of neoplasia, the features which are most usefully measured using flow cytometry are ploidy and cell surface marker phenotype. Neoplasms can be either diploid, i.e., with a normal DNA content or aneuploid, i.e., those which have other than a normal content. Aneuploidy usually correlates with tumor aggressiveness and a worse prognosis; however, in neuroblastoma hyperdiploid tumors have a better prognosis.[11]

Chemotherapy to targeted therapy

The prognosis for malignant solid tumors has improved since the introduction of effective chemotherapy capable of reducing the tumor volume and making previously unresectable tumors resectable. The operation also becomes safer and easier after pre-operative chemotherapy. Furthermore, there is no delay in treating metastatic disease, which is detectable at diagnosis in a significant proportion of patients.

Staging: Once the diagnosis has been confirmed, the extent of the tumor (size, position, relationship to surrounding structures, appearance of lymph nodes) must be established. Though there is no single uniform staging approach for childhood malignancies, the physician will need to be aware

of various investigations for staging of each tumor type according to the current protocols (Table III).

Increasingly, the chemotherapy response of the primary tumor in the post-surgical specimen is used in deciding post-operative treatment for a number of malignant solid tumors.

The effective use of cancer chemotherapy requires a thorough understanding of principles of neoplastic cell growth kinetics, basic pharmacologic mechanisms of drug action and pharmacokinetic and pharmacodynamic variability. The most common anticancer drugs are cytotoxic agents which are cell poisons that act indiscriminately on most cells, either causing direct damage to DNA or inhibiting cell replication. Cancer chemotherapy relies on exploiting the therapeutic index - the ratio of cell killing in the malignant cell population compared with killing of normal cells.

Multiagent therapy has three important theoretical advantages over single-agent therapy. Firstly, it maximizes the cell kill, while minimizing host toxicities by using agents with non- overlapping dose-limiting toxicities. Secondly, it may increase the range of drug activity against tumor cells with endogenous resistance to specific types of therapy. Finally, it may also prevent or slow the development of newly resistant tumor cells.

Adjuvant chemotherapy

Adjuvant chemotherapy is used after all of the known and visible cancer has been removed surgically or with radiation and should be given as soon as possible after definitive local therapy. A delay to allow for recovery from surgery or radiation therapy may compromise the chance of curing the patient. The aim is to prevent metastatic recurrence by eliminating micrometastatic tumor deposits in the lungs, bone, bone marrow, or other sites. It has been demonstrated to be efficacious for most of the common pediatric cancers, including Wilms' tumor, Ewing's sarcoma, osteosarcoma and rhabdomyosarcoma.

Increasingly, chemotherapy is used in a neo-adjuvant setting (before the definitive treatment) in pediatric solid tumors as chemotherapy shrinks the tumor and the operation becomes safer and easier. Neoadjuvant chemotherapy also provides earlier set treatment for micrometastases. The drive for all new therapies is to devise compounds that maximally target the tumor, avoiding systemic side effects.

Monoclonal antibody therapy

The era of monoclonal therapy has firmly arrived. Rituximab, a monoclonal antibody targeting cells expressing CD20 antigens, is licensed for use against follicular lymphoma and diffuse large B-cell non-Hodgkin's lymphoma (NHL).[12] Cetuximab is active against tumors expressing epidermal growth factor (EGFR). It has been used in adult practice against metastatic colorectal cancer and advanced squamous cell cancer in the head and neck.[13]

Table III. Investigations for staging of pediatric tumors

Germ cell	Wilms'	Neuro-blastoma	Lymphoma	Rhabdomyo-sarcoma	Hepato-blastoma	Osteo-sarcoma	Ewing's
AFP level	USS Abdo CT/MRI Abdo	Urinary Catecholamines	Bone marrow aspirate and trephine	CT/MRI scan local tumor	CT/MRI liver and abdo	MRI (or primary) before biopsy	CT or MRI (of primary) before biopsy
HCG	Chest X-ray; CT chest	Bone marrow aspirate/ trephine	CSF exam	CT chest; MRI abdo/ pelvis	MR angio-graphy; CT chest	CT chest	CT chest
MRI/CT; Abdo/chest		Bone/ MIBG scan; CT chest/ MRI abdo; Estimation of N-myc copy; +1p deletion from fresh tumor	CT chest; MRI of abdo/ pelvis; bone scan	CT/MRI brain scan (for head/ neck disease); Bone scan; Bone marrow; aspirate/ trephine	Bone marrow; aspirate/ trephine	Bone scan	Bone scan; bone marrow; aspirate/ trephine; fresh tumor for chromo- some analysis

Target therapy

Tyrosine kinase inhibitors act on genes that are responsible for many aspects of cell survival. These genes are important in cellular proliferation, differentiation, motility and apoptosis. Other targets that may be inhibited by small molecules include endothelial and vascular endothelial growth factors (EGF/VEGF) and once again evidence of expression has been found in cell lines in many pediatric tumors. Drugs targeting these pathways are currently undergoing phase I and II trials in the pediatric setting.

Cancer vaccination and T-cell therapy

Vaccination works by stimulating host T-cells to fight off disease. Anticancer vaccines have been worked on for many years and recent increased understanding of cellular biology has meant there have been crucial developments in producing useful anticancer vaccines. Vaccination strategy is not only dependent on optimizing antigen presentation but also the interaction of that presenting cell with disease-modulating T-cells. The most exciting results have been seen using patient-specific vaccines derived from autologous tumor cell lines. Melanoma, which increasingly

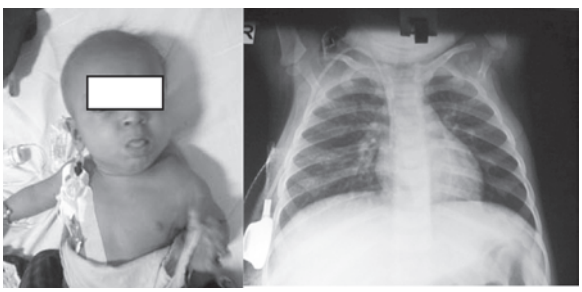

Fig.4. A child with cancer having portocath and X-ray showing it in position

affects teenagers and young adults, has shown the most susceptibility to a vaccination approach. A recent report of patient-specific dendritic cell vaccines in a cohort of heavily pretreated patients with metastatic disease, will hopefully prove to be a large step forward in the long search for a successful anticancer vaccine.[14]

Surgical procedures in pediatric cancer care

Primary surgical resection: In many cases of solid tumors, surgical excision of primary tumor is the preferred local treatment since radiotherapy has a much greater risk of long-term sequelae. The general principles of choice of local treatment are that surgical excision is the treatment of choice where: (i) complete excision is possible and results in improved survival and cure; (ii) it will give functional and cosmetic results better than those obtained by other treatment.

Central venous catheters and implantable devices: Insertion of central venous catheter is probably the single most frequent operation that pediatric surgeons perform while caring for a child with malignancy. Centrally placed, long-term venous catheters are used for the administration of chemotherapy, antibiotics and for blood sampling. Central venous catheters make care easier, both for the child and for the medical team. Currently there are two main types of catheters used in clinical practice–tunnelled, external catheters (Hickman®, Broviac®, Groshong®) and totally implanted access devices such as a portocath. External, tunnel catheters are generally easier to access, are less expensive than portocaths, offer less risk of extravasation into sub-cutaneous tissue, allow more rapid infusions, and can be removed easily at the end of treatment. However, the portocath offers an improved cosmetic result, less restriction in normal activities, less maintenance care and they are well protected, thus decreasing the chance of damage, and are associated with a lower risk of infection (Fig.4).

Feeding gastrostomy: Clinical experience has demonstrated that gastrostomy tubes are an effective way to deliver medications and to provide nutrition to children experiencing excessive emesis. Providing nutrition through a gastrostomy tube alleviates the frustration associated with forced feeding of the child via the mouth. Maintenance of normal patient nutrition throughout cancer treatment allows normal growth and improves quality of life.

Oncological complications: Surgeons may also be consulted to deal with complications related to other forms of treatment: extravasation of chemotherapy agents causing tissue necrosis, typhlitis (neutropenic enterocolitis), intestinal perforation, strictures or avascular necrosis, or other damage due to late effects of radiotherapy.

Surgical decisions, as well as those concerning chemotherapy, radiotherapy, and overall treatment strategies are best made after joint discussion, which is facilitated by a formal system of consultations such as regular multidisciplinary oncology team meetings (Tumor board), as well as maintaining communication between the key team members during the treatment.

Future directions

The result of the advances in knowledge of pediatric cancer is the need for all specialists to work together in an organized and coherent team approach. With increased

survival rates for childhood cancer, philosophy of treatment has changed over the years from 'Cure at any cost' to 'Cure at least possible cost'.

Surgeons, physicians and pathologists have a responsibility to ensure the supply and retention of tumor and normal tissue samples for research purposes if progress in diagnosis, prognostication and treatment is to be maintained. The application of RT-PCR and the generation of tissue microarrays is a revolution in cancer research with massive potential for early therapeutic gain. The role of diagnostic histopathology in the management of pediatric neoplasia is greater today than ever. The constant desire to improve survival with highly toxic therapies has led to a demand for a more detailed assessment of individual neoplasms in terms of specific histological types and their variants, stage and histological grade. The remarkable and rapidly accruing insights into the molecular biology and cytogenetics of tumors and tumorigenesis has not reduced the role of pathology. Rather it has imposed a need for pathologists to do more with tumor samples submitted for examination.

So what does the future hold? It is likely that gross disease will continue to be debulked by traditional treatment modalities. This may be followed by establishing a patient-specific, molecular tumor profile with microarray technology, allowing a targeted attack of disease residuum with small molecules, immunomodulation or vaccination.

Points to Remember

- ***Improved understanding of the molecular genetic basis of tumorigenesis has translated into diagnostic assays to identify abnormalities of gene or chromosome structure in patient tissues and as a means of supporting standard histopathologic and immunohistochemical diagnostic methods.***
- ***Advances in imaging have helped in better diagnosis and prognostication of pediatric solid tumors.***
- ***Targeted chemotherapy including monoclonal antibodies and adjuvant chemotherapy has revolutionized the treatment.***
- ***Advent of central venous lines to administer chemotherapy has made care of the child easier.***

References

1. Stiller AC. Epidemiology of Childhood Tumors. In: Surgery of Childhood tumors. Carachi R, Grosfeld JL, Azmy AF (eds) 2nd edn, Springer-Verlag Berlin Heidelberg, 2008;pp3-16.
2. Green DM, Jaffe N. Wilms' tumor-model of a curable pediatric malignant solid tumor. Cancer Treat Rev 1978; 5:143-172.
3. Davidoff AM, Krasin MJ. Principles of pediatric oncology/ genetics of cancer. In: Pediatric surgery Grosfeld JL, O'Neill JA, Coran AG (eds), 6th edn. Mosby, Philadelphia, 2006;pp411-436.
4. Brodeur GM, Maris JM, Yamashiro DJ, Hogarty MD, White PS. Biology and genetics of human neuroblastomas. J Pediatr Hematol Oncol 1997;19:93-101.
5. Hoffer FA. Magnetic resonance imaging of abdominal masses in the pediatric patient. Semin Ultrasound CT MR 2005;26:212-223.
6. Kellenberger CJ, Epelman M, Miller SF, Babyn PS. Fast stir whole-body MR imaging in children. RadioGraphics 2004;24:1317–1330.
7. Pashankar FD, O'dorisio MS, Menda Y. MIBG and somatostatin receptor analogs in children: Current con- cepts on diagnostic and therapeutic use. J Nucl Med 46 (Suppl) 2005;1:55s–61s.
8. Kilpatrick SE, Garvin AJ. Recent advances in the diagnosis of pediatric soft-tissue tumors. Med Pediatr Oncol 1999;32: 373-376.
9. Sailhamer E, Jackson CC, Vogel AM, Kang S, Wu Y, Chwals WJ, et al: Minimally invasive surgery for pediatric solid neoplasms. Am Surg 2003;69:566-568.
10. Partrick DA, Rothenberg SS. Thoracoscopic resection of mediastinal masses in infants and children: An evaluation of technique and results. J Pediatr Surg 2001;36:1165-1167.
11. Look AT, Hayes FA, Nitschke R, McWilliams NB, Green AA. Cellular DNA content as a predictor of response to chemotherapy in infants with unresectable neuroblastoma. New Engl J Med 1984;311:231-235.
12. Lynch DA, Yang XT. Therapeutic potential of ABX- EGA: A fully human anti-epidermal growth factor receptor monoclonal antibody for cancer treatment. Semin Oncl 2002;29:47-50.
13. Waksal HW. Role of anti-epidermal growth factor receptor in treating cancer. Cancer Metastases Rev 1999;18(4):427-436.
14. Dillman R, Dselvan F, Schiltz D. Patient-specific dendritic cell vaccines for metastatic melanoma. N Eng J Med 2006; 355:1179-1181.

HEMATO ONCOLOGY

PRIMARY IMMUNODEFICIENCY DISORDERS - WHEN TO SUSPECT AND HOW TO DIAGNOSE

***Revathi Raj**

Abstract: *Primary immune deficiency disorders are not as rare as thought and can affect about 1 in 10,000 live births. The diagnosis is often missed due to lack of awareness and can be made at any age starting from the newborn period to adulthood. Any child with recurrent infections, atypical organisms, unusual sites and refractory autoimmune disorder can have an underlying defect in their immune system. The introduction of flow cytometry based evaluation for these disorders has made rapid diagnosis a reality and has also given us good insight into the phenotype and genotype correlation. Awareness leads to early diagnosis and intervention with improved outcomes.*

Keywords: *Primary immunodeficiency, Recurrent infections, Flow cytometry, Atypical organisms, Autoimmunity.*

Genetic immune deficiency disorders hit the headlines with the case of the 'bubble baby disease'. Born in 1971 with severe combined immunodeficiency (SCID), David Vetter lived in a sterile bubble to avoid outside germs that could kill him. He underwent bone marrow transplantation from his sister. Advances in the field of immunology have made us understand our immune system with more clarity and have helped to introduce disease specific therapy individualized to each child. Autoimmunity is a feature of immune dysregulation and is often a feature of primary immune deficiency disorders. The Jeffrey Modell Foundation has created the 10 warning signs of primary immune deficiency to raise awareness amongst primary care physicians and has been an effective campaign. There have been numerous advances in the field of diagnostics and therapy for these children. Outcomes are based on early diagnosis and referral to the nearest tertiary care centre. Several states in the USA have also introduced newborn screening for severe combined immune deficiency disorders resulting in close to 100% survival in these children.[1]

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Pointers for primary immune deficiency

The European society for primary immune deficiency (PID) disorders has suggested the ten warning signals (Box 1) to suspect and investigate a child with such a disorder.[2]

Box 1. Ten warning signals for PID

- Four or more new ear infections within 1 year.
- Two or more serious sinus infections within 1 year.
- Two or more months on antibiotics with little effect.
- Two or more pneumonias within 1 year.
- Failure of an infant to gain weight or grow normally.
- Recurrent, deep skin or organ abscesses.
- Persistent thrush in mouth or fungal infection on skin.
- Need for intravenous antibiotics to clear infections.
- Two or more deep-seated infections including septicemia.
- A family history of PID.

In our country, poverty, malnutrition, overcrowding and rampant tuberculosis in the community often mislead a clinician and the diagnosis of PID is therefore delayed. As consanguinity rates are high, autosomal recessive forms of PID are also more prevalent. A child with PID may present to the neonatologist with severe sepsis, pediatric surgeon with a non-healing ulcer or granulomatous abscesses, pediatric intensivist with fulminant pneumonia, pediatric gastroenterologist with recurrent diarrhea and failure to thrive, rheumatologist with recurrent arthritis, hematologist with immune cytopenia, oncologist with early onset lymphoma, genetic counsellor for recurrent early infant deaths or to a nephrologist with nephritis.

Classification

PID is classified based on the defect at the cellular

Table I. Classification of primary immunodeficiency disorders

Defects	Examples
T cell defects	Severe combined immune deficiency (SCID), Wiskott Aldrich syndrome (WAS), Hyper Ig M syndrome
B cell defects	X-linked agammaglobulinemia (XLA), Common variable immunedeficiency (CVID)
Phagocytic defects	Neutrophil function defects:Leucocyte adhesion defect (LAD), Chronic granulomatous disease (CGD)
Immune dysregulation	Hemophagocytic lymphohistiocytosis (HLH), Autoimmune lymphoproliferative syndrome (ALPS)
Complement deficiencies	C5 to C9

level.[3] The common types of PID with some examples are given in Table I.

T cell defects: These children present early with atypical infections like pneumocystis jiroveci pneumonia, disseminated BCG and cytomegalovirus infections. Failure to thrive is a hallmark of all these PIDs.[4] Mendelian susceptibility to mycobacterial disease (MSMD)is a distinct T cell disorder with recurrent tuberculosis.

B cell defects: These children present with recurrent bacterial infections and respond to antibiotics. X Linked agammaglobulinemia (XLA) presents in infancy and Combined variable immunodeficiency (CVID) later in life.[5,6]

Phagocytic defects: LAD often presents in infancy with recurrent abscesses filled with serosanguinous fluid rather than pus as the neutrophils required to make pus do not migrate to the site of infection. Delayed umbilical cord separation and non-healing ulcers in an infant should also raise the suspicion of LAD.[7] Children with CGD present with recurrent granuloma or abscesses due to staphylococcal infection.[8]

Immune dysregulation: Hemophagocytic lymphohistiocytosis (HLH) is a disorder of the monocyte - macrophage system which can present in early infancy with high grade fever, pancytopenia and hepatosplenomegaly. The histiocyte society has recommended criteria for diagnosis (Box 2).[9] HLH can be associated with syndromes such as Griscelli syndrome and Chediak Higashi syndrome. Associated partial albinism will offer a diagnostic clue.

Autoimmune lymphoproliferative syndrome (ALPS) is another disorder resulting from immune dysregulation.[10]

Box 2. HLH diagnostic criteria

Molecular diagnosis of hemophagocytic lymphohistiocytosis (HLH) or X-linked lymphoproliferative syndrome (XLP)

Or

Three of these four criteria:

1. Fever ≥38.5°C
2. Splenomegaly
3. Cytopenias (minimum 2 cell lines reduced)
   - Hemoglobin < 9g/dL
   - In infants < 4 weeks - Hemoglobin < 10g/dL
   - Platelets < 100×10^3/mL;
   - Neutrophil < 1×10^3/mL
4. Hepatitis

And

At least 1 of 4:

1. Hemophagocytosis in bone marrow or spleen or lymphnodes
2. Ferritin > 500ng/mL
3. Elevated soluble CD 25 [i.e.,soluble interleukin-2 receptor alpha (sIL-2Rα) (age-based)]
4. Absent or very low NK function

Other results supportive of HLH diagnosis:

a. Hypertriglyceridemia (>265 mg/dL)
b. Hypofibrinogenemia (<150 mg/dL)
c. Hyponatremia

T lymphocytes migrate to the thymus and need to get programmed to become either helper or cytotoxic T lymphocytes after acquiring either CD4 or CD8 antigens respectively. If the lymphocytes emerge out of the thymus with neither antigen they are called double negative T cells. These cells cause rampant autoimmunity with lymph node hyperplasia, splenohepatomegaly and pancytopenia with high fever.

Complement defects: These comprise 1%-10% of all primary immunodeficiencies.[11] The genetic deficiency of early components of the classical pathway (C_1q, C_1r/s, C_2, C_4) tend to be linked with autoimmune diseases whereas C_5 to C_9 deficiencies often present with recurrent meningococcal meningitis or with nephritis.

Relevance of the host and pathogen features

Host features such as eczema and thrombocytopenia point to Wiskott Aldrich syndrome. The presence of generalized lymphadenopathy and polyclonal increase in T lymphocytes is the hallmark of Omenns syndrome. Dysmorphism with coarse facies and mid-facial defects are seen in Hyper Ig E syndrome. Cryptosporidium induced diarrhea is a hallmark infection in hyper Ig M syndrome as is Burkholderia sepsis in CGD.

Diagnosis

Investigation a child with suspected immune deficiency begins with a complete blood count.

Box 3. Flow cytometry

1. Lymphocyte subset analysis	
T Lymphocytes	CD3
Helper T cells	CD4
Cytotoxic T cells	CD8
B lymphocytes	CD19
Natural Killer cells	CD56
2. Phagocytic function analysis	
CGD	Dihydrorhodamine (DHR) assay
LAD	CD11/18
3. Immune dysregulation	
ALPS	CD 4/ CD8 negative (Double negative) T cells greater than 2.5%
HLH	Soluble CD25/NK cytotoxicity

The absolute neutrophil count and absolute lymphocyte count should be at least 1000/mm^3 in children of all age groups including neonates. Neutropenia is often associated with Hyper IgM syndrome and thrombocytopenia is seen in Wiskott Aldrich syndrome. Neutrophilic leucocytosis is seen in LAD and CGD. There are inclusion bodies in the neutrophils in children with Chediak Higashi syndrome. Complement deficiency is diagnosed by complement assay. Flow cytometry based assays are the standard means of diagnosing PID (Box.3)[12]

Genetic testing

Gene mutation analysis has provided deeper insights into the molecular pathogenesis of each of the PID disorders. Flow cytometry can provide an accurate prediction of the possible gene mutation in SCID (Box 4).

Box 4. Gene mutations

T negative, B positive, NK negative	Gamma chain defect
T negative, B negative, NK positive	RAG mutation
T negative B negative, NK negative	ADA deficiency

This information helps us plan transplantation as some types require conditioning chemotherapy before hematopoietic stem cell transplant (HSCT) and others may benefit from stem cell infusion without chemotherapy. However, if the diagnosis is difficult or the couple wishes to plan another pregnancy gene mutation analysis is essential. Prenatal diagnosis can be done using the gene mutation analysis at 11 weeks. Alternatively a cordocentesis sample obtained at 20 weeks can be analysed using flow cytometry and accurate diagnosis can be provided.

Management

Children with PID need to be stabilized as they often arrive with multiple infections. Nutritional support and the use of leucodepleted and irradiated blood products are recommended. Currently, hematopoietic stem cell transplantation. (HSCT) is the main curative option.[13, 14] Children with T cell defects are offered HSCT and if feasible, gene therapy or ADA enzyme replacement. The best outcomes are seen when the baby is infection-free and a fully HLA matched family donor is available for the HSCT. Children with B cell defects need long term immunoglobulin replacement every 28 days at 400 mg/kg/dose. Children with phagocyte disorders are stabilised with cotrimoxazole and itraconazole prophylaxis and then referred for an elective HSCT. HLH is treated with

chemotherapy as per HLH2004 protocol followed by HSCT. Children with ALPS are treated with long term immunosuppression with sirolimus.

Conclusion

The spectrum of PID is complex and every individual disease and child needs tailored therapy to ensure optimal outcomes. Early diagnosis before the onset of serious infection makes a tremendous difference in reducing the morbidity and mortality. Genetic counselling and prenatal diagnosis must be offered in all cases of PID to prevent the birth of another affected baby in the same family.

Points to Remember

- ***Primary immune deficiency disorders can manifest at any age from the newborn period to adulthood and diagnosis is feasible only if there is adequate awareness.***
- ***Any child with recurrent or unusual infections or refractory autoimmunity should be evaluated for a defect in the immune system.***
- ***Flow cytometry based evaluation for T and B cell markers and serum immunoglobulins form a basic screening test for these children.***
- ***Hematopoietic stem cell transplantation (HSCT) is the main curative option in many of the primary immune deficiency disorders.***

References

1. Puck JM. Neonatal screening for severe combined immunodeficiency. Curr Opin Pediatr 2011;23:667-673.
2. de Vries E, Driessen G. Primary immunodeficiencies in children: a diagnostic challenge. Eur J Pediatr 2011; 170: 169–177.
3. Al-Herz W, Bousfiha A, Casanova JL, Chapel H, Conley ME, Rundles CC, et al. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency. Front Immun 2011;2:54.
4. Subbarayan A, Colarusso G, Hughes SM, Gennery AR, Slatter M, Cant AJ, et al. Clinical features that identify children with primary immunodeficiency diseases. Pediatrics 2011;127:810-816.
5. Slatter MA, Gennery AR. Clinical immunology review series: an approach to the patient with recurrent infections in childhood. Clin Exp Immunol 2008;152:389-396.
6. Wood P, Stanworth S, Burton J, Jones A, Peckham DG, Green T, et al. UK Primary Immunodeficiency Network. Recognition, clinical diagnosis and management of patients with primary antibody deficiencies: a systematic review. Clin Exp Immunol 2007;149:410-423.
7. Madkaikar M, Currimbhoy Z, Gupta M, Desai M, Rao M. Clinical profile of leukocyte adhesion deficiency. Indian Pediatr 2011;49:1-4.
8. Holland SM. Chronic granulomatous disease. Clin Rev Allergy Immunol 2010;38:3-10.
9. Henter J, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2006;48:124-131.
10. Madkaikar M, Mhatre S, Gupta M, Ghosh K. Advances in autoimmune lymphoproliferative syndromes. Eur J Haematol 2011;87:1-9.
11. Grumach AS, Kirschfink M. Are complement deficiencies really rare? Overview on prevalence, clinical importance and modern diagnostic approach. Mol Immunol 2014;61:110-117.
12. Madkaikar M, Mishra A, Ghosh K. Diagnostic Approach to Primary Immunodeficiency Disorders. Indian pediatr 2013;50:579-586.
13. Notarangelo LD, Forino C, Mazzolari E. Stem cell transplantation in primary immunodeficiencies. Curr Opin Allergy Clin Immunol 2006;6:443-448.
14. Gaspar HB, Qasim W, Davies EG, Rao K, Amrolia PJ, Veys P. How I treat severe combined immunodeficiency. Blood 2013;122:3749-3758.

ERRATUM

Article titled "Fetal choletithiasis – A follow up" Indian J Pract Pediatr 2016; 18(2): 212 - 214

It is regretted that the designation of co-author 'Dr.W.K.Vindyarani' designation was wrongly printed in the above article. The authorship of the article should read as "Vindyarani WK, Professor and Head. The error is deeply regretted.

Editorial Board
Indian Journal of Practical Pediatrics

GENERAL ARTICLE

CHIKUNGUNYA IN CHILDREN

***Pravakar Mishra**
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Abstract: *Chikungunya is a viral infection spread by the mosquito belonging to the Aedes species. The disease has been occurring in epidemic forms in our country over the past two decades. Unlike adults, the affected children have less of musculoskeletal involvement, but more of fever with skin rash and may also present with febrile seizures. Children also may have neurological manifestations, which are rare but severe, with sequelae. Perinatal chikungunya due to maternal infection can result in neonatal fever, rash, edema, neurologic problems and multiorgan failure. Treatment of chikungunya is symptomatic. Preventive strategies include control of mosquito breeding and personal protection against mosquito bites.*

Keywords: *Chikungunya, Children, Neurological, Mother-to-child transmission.*

Chikungunya is a viral fever spread by mosquito Aedes species (A aegypti, A albopictus). The virus responsible is an alphavirus chikungunya virus (CHIKV).[1] In the year 1952-53, the first outbreak of the disease occurred in the southern part of Tanzania, after this it was named as chikungunya, the name being derived from the Makonde language of Tanzania which means "that which bends up" because of the severe arthralgia leading to inability to stand or walk.[2] Since the last decade, the disease is being increasingly reported from different parts of India in epidemics drawing increased attention.

Epidemiology

Before its detection and nomenclature in 1952-53, epidemics of fever and polyarthralgia were noted to occur in major parts of Africa, Asia, Southern America and West Indies. It was wrongly interpreted as dengue till the isolation of chikungunya virus in 1952-53 during an epidemic. Since then, the disease has been reported as outbreaks in different regions of the world. Early epidemics were reported in some regions of Africa and Asia-Pacific from year 1952 to 2000.[1,3] In the year 2004, it spread to the east coast of Africa, islands of the Indian Ocean, India, Southeast Asia and Pacific affecting millions of people. First epidemic was reported from India in early 1960s into the 1970s.[4]A high attack rate was observed during the epidemic in Chennai (40%) in the year 1962 to 1964 and in Barsi, Maharashtra (37%) in the year 1973.[5] Thereafter, no report of epidemics occurred in India till early 21st Century.[6] The disease has reemerged after the early epidemics probably because of increased travelers across the globe, population explosion and urbanization leading to increase in mosquito breeding sites and improper vector control measures. This explanation holds true considering the recent rise in dengue epidemics in different parts of India and rest of the world, as the vector transmitting both the diseases are same.

Etiopathogenesis

Chikungunya virus (CHIKV) is a positive-sense, single-stranded RNA virus of genus Alphavirus (Togaviridae family). It is transmitted by mosquito vectors that are anthropophilic and belong to Aedes species (A.aegypti, A.albopictus and A.*henselli*). As humans are not the dead end hosts for CHIKV (rather they help in transmission of the viruses through the mosquito vector), rapid mosquito-human-mosquito transmission cycle occurs leading to massive outbreaks. Unlike A.aegypti which is primarily found in tropical and sub-tropical regions, A.albopictus (Asian tiger mosquito) primarily a native to Asia subcontinent has spread globally because of capability of thriving in temperate regions also.[7] Another Aedes species (A *henselli)* has been shown to cause outbreaks in some regions of the world.

CHIKV comprises of 3 major genotypes: East Central South African (ECSA), West African, Asian plus Indian Ocean lineage (IOL) - a subtype of ECSA.[8] The outbreaks now occurring worldwide are being caused by both ECSA and Asian genotypes. After the bite of an infected female

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mosquito, the virus enters into the dermis and replicates in the connective tissue, epithelial cells and fibroblasts. Circulating monocytes are responsible for dissemination into the bloodstream during the viremic phase in the first 5-7 days. Secondary infection of fibroblasts occurs in muscles and joints. The virus is also detected in the epithelial and endothelial cells of various other organs. There is a strong type I interferon response by infected fibroblasts and other cell types during acute phase (first week of illness) which is prolonged in adults and behaves like chronic arthritis. Adaptive immunity against CHIKV is less well understood, develops after the first week when viral replication has been limited by innate immunity. CHIKV specific immunoglobulins protect against infection, but both B and T cells may contribute to pathogenesis and long term musculoskeletal manifestations.[9]

The ECSA virus (containing the E1: A226V mutation) spread to India by 2006, when large number of cases were reported in the first year of the epidemic itself.[10] The activity is still continuing in India after this, till now. From India, the strain spread to Southeast Asia and to Northern Italy.

Clinical features

The median incubation period is 2 to 4 (range 1-12) days. There is a sudden onset fever, severe arthralgia, headache, photophobia and skin rash.[1] Fever is typically high-grade in both children and adults.

Children have different clinical presentations than adults because of the rate of susceptibility, development of proper immune system, maturity of different organ systems, etc. The diversity and severity of symptoms resembles a U-shaped curve (being maximum in young infants and the elderly, minimum in older children). In children, febrile seizures frequently occur beyond the typical age range of 6 months to 6 years.[11,12] Sometime, they may be misdiagnosed as atypical febrile seizure leading to long-term initiation of anti-epileptic drugs. A good percentage of infection in children are asymptomatic, the rate varying from 35% to 40% in different outbreak reports.

A variety of skin manifestations occur in chikungunya fever. These include skin pigmentary changes in the centrofacial area, maculopapular rash and intertriginous aphthous-like ulcers.[11,12] Skin rash usually lasts for 5 days, with hyper-pigmentation sometimes following the rash. Infants below 6 months may exhibit extensive bullous skin lesions with blistering.[13] Hemorrhagic manifestations including epistaxis, gingival bleeding and purpura are observed in less than 10% of children.

Musculoskeletal manifestations including myalgia, arthralgia and arthritis are common in adults, but less common in children.[11] Finger, wrist, ankle, elbow and knee joints are commonly affected.[1] Swelling without other signs of synovitis is typically reported with a symmetric, distal, polyarticular pattern. Permanent arthropathy of affected joints is rare. Other manifestations include tenosynovitis, tendinitis or bursitis during acute and sub-acute stages. There is a chance that the arthralgia may persist for years particularly in adults.

Neurological manifestations are rare but may be more severe. Central nervous system (CNS) involvement potentially is more significant than previously documented, especially in children. Though reported at a higher figure (25%) outside India, in India, 14% of all children presenting with suspected CNS infection had chikungunya.[14,15] Of them, a high proportion (40%-50%) had severe manifestations, including status epilepticus, complex febrile seizures and encephalitis. The incidence of encephalitis was significant in those younger than 1 year of age, as well as those older than 45 years. Though mortality is rare, in La Réunion, 9% of patients with neurological manifestations died. Long-term neurological symptoms have been reported in both children and adults in a La Réunion study that included more than 1000 individuals (28% being children).[14] Two years after acute infection, cerebral disorders (including attention and memory difficulties) were reported in approximately 75% subjects, and sensory-neural disorders (including blurred vision and hearing difficulties) in nearly 50%. These findings clearly show that chikungunya in children is not always a benign infection. Rather, it may result in long-term sequelae.[2]

Perinatal infection

It was first described during the outbreak in La Réunion in the year 2005.[15] Although intrauterine transmission was absent or very rare in early pregnancy, it rose to nearly 50% when mothers were viremic in the week just preceding delivery. Infected neonates developed symptoms around day 4 (range 3-7) of life. Most common signs were fever, rash and edema. Others included petechiae, thrombocytopenia and lymphopenia. Complications included intra-cerebral hemorrhages, status epilepticus and multi-organ failure, which necessitated mechanical ventilation in around 25% of the neonates.[13] The long-term outcome of survivors was poor as half of them had diminished neurocognitive performance at 2 years of age.[16]

Diagnosis

Chikungunya should be suspected when a child presents with high-grade fever of acute onset, rash and/or arthralgia and/or edema not explained by other causes. A chikungunya diagnosis is likely if the child has visited or lived in an endemic area. However, it is important to keep in mind that cases may appear in places where chikungunya is not endemic. Besides routine blood tests (complete blood counts, and tests for malaria and other common infections to rule out the diagnosis), virological and serological tests are necessary for laboratory confirmation of index case. During the first 5 days of infection, the virus can be found in the blood by quantitative [reverse transcriptase polymerase chain reaction (RT-PCR)]. ELISA test may confirm the presence of virus specific IgM/IgG antibodies. IgM antibodies appear between 2-7 days after onset of disease, whereas IgG antibodies frequently are detected after the first week of illness. WHO recommends both serological and virological testing of samples collected during the first week after onset of symptoms.

The differential diagnosis-includes malaria, dengue, typhoid fever, influenza, hepatitis, leptospirosis and rickettsial infection. In addition, in areas where these viruses are present, infection with West Nile virus and other viruses belonging to the group of Flavivirus, Togavirus, Bunyavirus and Reoviruses should be considered. Dengue is the infection most capable of mimicking chikungunya. In this regard, clinical signs including arthralgia and rash cannot be reliably used to distinguish dengue and chikungunya.[17] However, rash appears earlier in the course of chikungunya than it does with dengue. Furthermore, though thrombocytopenia is more frequently seen in patients with dengue, up to 50% of children with chikungunya also have mild thrombocytopenia.[17]

Treatment

There is no specific treatment for chikungunya. Management is therefore symptomatic and focuses mostly on adequate hydration, antipyretics and analgesics. Paracetamol at 15mg/kg/dose is used for relief from fever/pain. Salicylates and non-steroidal anti-inflammatory drugs (NSAIDs) should be carefully used, as these may precipitate bleeding manifestations if platelet counts are low.[11] Although the symptoms of patients with persistent joint pain may be challenging to manage, NSAIDs together with corticosteroids or hydroxychloroquine / methotrexate successfully have been used in adults. Ribavirin also has been shown to improve chronic arthralgia / arthritis in some adult patients, but its benefit in acute pediatric infection remains unknown.[1]

Prevention/Control

Eradication of the vector: Historically, the prevention/control of mosquito-transmitted viruses has been dependent upon efforts aimed at control of mosquito populations. These activities focus on elimination of mosquito larval habitat and prevention/control of human bite. Because *Aedes* mosquitoes are container-breeding species, control of larval habitat is challenging, though not impossible. Prevention/control efforts aimed at reducing human biting populations also fail, because the large scale pesticide applications do not reach many that bite indoors. Indoor pesticide spraying might be effective, but is impractical in large urban areas.

Vaccines: In the year 2000, a live-attenuated vaccine was tested in a randomized, double-blind, placebo-controlled trial. Seroconversion rates were high (98%), but the vaccine temporally was associated with arthralgia in 8% of vaccinees.[1] More than 20 candidate vaccines are under development, some are in phase I and II trials.

Personal protection against mosquito bites: As with malaria, appropriate clothing minimizes exposure to mosquito bites. It should be remembered that Aedes mosquitoes bite during day-time. Insect repellents containing N, N-diethyl-3-methylbenzamide, IR3535 or icaridin should be applied to exposed skin. In addition, insecticide-treated mosquito nets are vital for infants and children who sleep during the daytime.

Conclusion

Rapid urbanization, increased travel across continents, viral / vector adaptation and lack of effective control measures probably have contributed to the recent outbreaks of chikungunya. Like other mosquito-borne diseases, it is difficult to predict when an outbreak is going to occur in a particular location. Management is entirely symptomatic. While vaccine seems to be an attractive control measure it has to go through further trial phases before being put into clinical use.

Points to Remember

- ***Chikungunya is a re emerging viral infection spread by Aedes mosquitoes.***
- ***Like other mosquito-borne diseases, it is difficult to predict when an outbreak is going to occur in a particular location.***

- ***Children present commonly with fever and rashes. Musculoskeletal manifestions are rare compared to adults.***
- ***Mother-to-child transmission can occur with neonates presenting with fever, rash, edema and neurological problems.***
- ***Management is entirely symptomatic.***
- ***Mosquito breeding control and prevention of mosquito bites are the only currently available preventive strategies.***

References

1. Burt FJ, Rolph MS, Rulli NE, Mahalingam S, Heise MT. Chikungunya: a re-emerging virus. Lancet 2012;379: 662-671.
2. Kumar A, Best C, Benskin G. Epidemiology, Clinical and Laboratory Features and Course of Chikungunya among a Cohort of Children during the First Caribbean Epidemic. J Trop Pediatr 2016 Aug 10.pii: fmw051. [Epub ahead of print]
3. Powers AM, Logue CH. Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. J Gen Virol 2007;88: 2363-2377.
4. Myers RM, Carey DE. Concurrent isolation from patient of two arboviruses, Chikungunya and dengue type 2. Science 1967;157: 1307-1308.
5. Padbidri VS, Gnaneswar TT. Epidemiological investigations of chikungunya epidemic at Barsi, Maharashtra state, India. J Hyg Epidemiol Microbiol Immunol 1979;23: 445-451.
6. Lahariya C, Pradhan SK. Emergence of chikungunya virus in Indian subcontinent after 32 years: A review. J Vector Borne Dis 2006;43: 151-160.
7. Petersen LR, Powers AM. Chikungunya: epidemiology. F1000 Res. 2016 Jan 19;5. pii: F1000 Faculty Rev-82. doi: 10.12688/f1000research.7171.1. eCollection 2016.
8. Mudurangaplar B, Peerapur BV. Molecular Characterisation of Clinical Isolates of Chikungunya Virus: A Study from Tertiary Care Hospitals in Southern India. J Clin Diagn Res 2016;10:DC14-17. doi: 10.7860/JCDR/2016/18370.7509. Epub 2016 Mar 1.
9. Schwartz O, Albert ML. Biology and pathogenesis of chikungunya virus. Nat Rev Microbiol 2010;8:491-500.
10. Ray P, Ratagiri VH, Kabra SK, Lodha R, Sharma S, Sharma BS, et al. Chikungunya infection in India: results of a prospective hospital based multi-centric study. PLoS One 2012;7:e30025. doi: 10.1371/journal.pone.0030025. Epub 2012 Feb 17.
11. Sebastian MR, Lodha R, Kabra SK. Chikungunya infection in children. Indian J Pediatr 2009;76: 185-189.
12. Ernould S, Walters H, Alessandri JL, Llanas B, Jaffar MC, Robin S, et al. [Chikungunya in paediatrics: epidemic of 2005– 2006 in Saint-Denis, Reunion Island]. Arch Pediatr 2008;15: 253-262.
13. Robin S, RamfulD, Zettor J, Benhamou L, Jaffar-Bandjee MC, Rivière JP, et al. Severe bullous skin lesions associated with Chikungunya virus infection in small infants. Eur J Pediatr 2010;169:67-72.
14. Robin S, Ramful D, Le Seach' F, Jaffar-Bandjee MC, Rigou G, Alessandri JL. Neurologic manifestations of pediatric chikungunya infection. J Child Neurol 2008;23: 1028-1035.
15. Ramful D, Carbonnier M, Pasquet M, Bouhmani B, Ghazouani J, Noormahomed T, et al. Mother- to-child transmission of Chikungunya virus infection. Pediatr Infect Dis J 2007;26: 811-815.
16. Gérardin P, Sampériz S, Ramful D, Boumahni B, Bintner M, Alessandri JL, et al. Neurocognitive outcome of children exposed to perinatal mother-to-child Chikungunya virus infection: the CHIMERE cohort study on Reunion Island. PLoSNegl Trop Dis 2014;8: e2996.
17. Laoprasopwattana K, Kaewjungwad L, Jarumanokul R, Geater A. Differential diagnosis of Chikungunya, dengue viral infection and other acute febrile illnesses in children. Pediatr Infect Dis J 2012;31: 459-463.

NEWS AND NOTES

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DRUG PROFILE

SUPPOSITORIES IN PEDIATRIC THERAPEUTICS

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Abstract: *Generally, oral administration is the route of choice for medicating children. However, suppositories are considered as a practical alternative when oral administration is either impractical or impossible, when there is vomiting, convulsions, non-cooperation, unconsciousness and in perioperative period. This article reviews the mechanism of absorption of suppositories, various formulations available and the advantages and disadvantages of some of the medications.*

Keywords: *Suppositories, Formulation, Rectal drug delivery, Children*

Absorption through the rectal route

Absorption of a drug from the rectal route depends on the composition of the rectal formulation. It varies with the nature of the suppository base used, which can be solid or liquid. The difference in rate of absorption in different suppository bases has been clearly demonstrated for drugs like chloral hydrate, diazepam, paracetamol, indomethacin and methadione.[1] An ideal suppository base should be non-toxic, non-irritant to the mucous membrane, melts, dissolves in rectal fluids and be stable on storage, compatible with many drugs, i.e. it should not bind with the drug or otherwise interfere with its release or absorption.

The following bases are commonly used for rectal suppositories. The fatty base is the traditional cocoa butter vehicle. It is immiscible in aqueous body fluids but melts at body temperature. Fatty base is used for hydrophilic drugs. The other base is the water soluble or water miscible base which is made of polyethylene glycol polymers and is used for lipophilic drugs. A third variety of base used are known as hydrogels which are used for controlled release rectal drug delivery formulation as required for drugs like antipyrene and theophylline.[2]

The rate and extent of rectal drug absorption is often lower than oral absorption because of an usually empty rectum, lack of active motility in resting position, inert mucous fluid with no enzymatic or buffering property, absence of villi or microvilli and the small absorptive surface area of 200-400cm^2.

There are some drugs in which the extent of rectal absorption is reported to exceed oral values. This could be due to the avoidance of hepatic first pass metabolism after rectal drug delivery resulting in direct delivery of drug to the systemic circulation. This phenomenon has been reported for drugs like morphine, metoclopromide, ergotamine, lidocaine and propranolol.[3] The extent of first-pass metabolism may be influenced, depending on the site of drug administration in the rectum (lidocaine). The rate of delivery may determine systemic drug action and side effects (nifedipine), and it may affect the local action of concurrently administered absorption promoters on drug uptake (cefoxitin).[1] Rectal drug delivery in a site and rate-controlled manner using osmotic pumps or hydrogel formulations may help in changing systemic drug concentrations and drug effects.

Types of rectal formulations

These include rectal semisolid preparations like creams, gels, ointments and suppositories, rectal liquid preparations like solutions and suspensions and rectal aerosols.

Drugs that can be used as suppositories[4]

There are a number of suppositories listed in British National Formulary (BNF) for use in children. These include glycerol, bisacodyl, morphine salts, mesalazine, ondansetron, ergotamine, meloxicam, carbamazepine, cyclizine, chloral hydrate, chlorpromazine, domperidone, indomethacin, sulfasalazine, paracetamol, prednisolone, metronidazole, sodium picosulphate, phosphates, diclofenac sodium, aspirin, compound anal and rectal preparations with corticosteroids.[5] The list of suppositories available in India with their strengths is shown in Table I.

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Table I. Suppositories available in India and their strengths

Drug	Strength
Glycerol	140mg, 700mg
Bisacodyl	5mg, 10mg
Paracetamol	80mg, 170mg, 250mg
Diclofenac	12.5mg, 25mg, 100mg
Diazepam	2.5mg, 5mg
Domperidone	30mg
Mesalazine	250mg
Sulfasalazine	500mg
Carbamazepine	125mg, 250mg
Morphine salts	5mg, 10mg, 20mg, 30mg
Prednisolone	5mg
Buscopan	7.5mg

Problems with rectal drug administration

Some of the side effects of the suppository administration per se include local irritation and rectal ulceration as in long term use of ergotamine and acetyl salicyclic acid. The assessment of tolerability and safety is imperative in the design of rectal formulations.[1]

Contraindications to use rectal suppositories

These include conditions like acute surgical abdomen, intestinal obstruction/perforation, paralytic ileus and anal or rectal fissures.

Paracetamol suppository

Dosage of paracetamol suppository for various age groups is given in Table II.

Comparison between oral and rectal paracetamol

Generally there is a trend among pediatricians to give paracetamol rectally to bring down the temperature fast in case of high fever. However, studies have proved that there is no difference between the temperature decrement in patients treated with 15mg/kg oral paracetamol and the same or double the dose rectally. Thus there seems to be no evidence to support the use of higher doses of rectal paracetamol for the treatment of fever in children.[6,7]

Comparison of oral versus rectal paracetamol with codeine in postoperative pediatric adenotonsillectomy patients: Suppositories achieved equivalent pain control as oral medication with few side effects and good tolerance. Many parents preferred the suppositories to oral for post operative pain relief in view of ease of administration. If given the choice for future surgeries, many parents would switch or consider switching from oral pain medications to suppositories.[8]

Diazepam suppository

These suppositories would seem to be of little value in treatment of acute febrile convulsions though the solution form is useful.[9] Solution is rapidly absorbed into the systemic circulation to achieve serum concentration >200ng/mL in 10min. Suppositories are absorbed slowly and hence not recommended for emergency management of seizures.[10] It has been shown that suppositories after a febrile seizure reduces the incidence of recurrent febrile seizures during same febrile illness.[11] Both rectal and IV diazepam preparations are equally effective and safe in

Table II. Paracetamol suppository[5]

Age	Dose*
1-3 months	30mg/kg as a single dose then 15-20mg/kg every 4-6 hours
3months- 6years	30-40mg/kg as a single dose; 15-20mg/kg every 4-6 hours
6- 12 years	30-40mg/kg (maximum 1g) as a single dose; 15-20mg/kg every 4-6 hours
12-18 years	1g every 4-6 hours (maximum 4 doses in a day)

*Maximum 90mg/kg/day in divided doses

cessation of seizures in children with intractable epilepsy.[12] The dosage is 0.2-0.7 mg/kg and available strengths are 2.5 mg and 5 mg.

Bisacodyl suppository

It is used for the management of constipation in children. This when used for preparation for sigmoidoscop affords swift and sure clearance of lower bowel without producing any alteration in appearance of mucosa. It also has a role in treatment of idiopathic megarectum in children.[13,14] The dosages are 5mg for <10yr and 5-10 mg for >10yr.

Glycerine suppository

This is useful in the management of constipation. It is a hyperosmotic laxative which irritates the inner lining of intestine and causes an excess flow of water in the intestine which makes the stool soft and initiates bowel movement within few minutes. The suppository should be inserted just half an inch to the baby's rectum.[15] It is administered by moistening with water before insertion. Dosages: Adults and children over 12 years: one 4g suppository; children 12 months to 12 years: one 2g suppository; newborns to 12 months: one 1g suppository.

Mesalazine suppository (5-aminosalicylic acid)

Daily bed time 500mg mesalazine suppository is found to be safe and effective in children with ulcerative proctitis.[16] Mesalazine suppositories produce earlier and significantly better results than oral mesalazine in the treatment of active ulcerative proctitis.[17] The dosage for children 12-18yrs is 250-500 mg 3 times daily, with last dose at bedtime or 1gm daily for 2-4 weeks for an acute attack followed by maintenance.

Sulfasalazine suppository

It can be used in the treatment of mild to moderate or severe ulcerative colitis and in the maintenance of remission. Areas under the concentration-time curves (AUC) and C_{max} are significantly lower after rectal than oral administration resulting in lower frequency of side effects.[18] The dosage for a child 5-8yrs is 500mg twice daily, 8-12yrs is 500mg in the morning and 1gm at night, 12-18yrs is 0.5-1gm twice daily.

Artesunate suppository

It is frequently used especially in African countries, especially when there is severe malaria which cannot be treated orally and where access to injection takes several hours. A single inexpensive artesunate suppository at the time of referral subsequently reduces the risk of death or permanent disability.[19, 20]

Carbamazepine suppository

One should know that 25% higher dose of carbamazepine should be given when given rectally.[21] 125mg may be considered to be approximately equivalent in therapeutic effect to tablets of 100mg. Final adjustment should always depend on clinical response and plasma concentration monitoring is required. The maximum dose by rectum is 250mg 4 times a day. It can also be used for short term (maximum 7 days) when oral therapy is temporarily not possible.

Morphine salt suppositories

Dosage of morphine suppositories for various age groups[22] is given in Table III.

Table III. Dose of morphine suppository

Age	Dose*
1-3 months	Initially 50-100mcg/kg Q4H
3- 6 months	100-150mcg/kg Q4H
6 months-1 year	200mcg/kg Q4H
1-2yrs	200-300mcg/kg Q4H
2-12yrs	200-300mcg/kg (max 10mg) Q4H
12-18yrs	5-10mg Q4H

*Adjusted to response

Domperidone suppository[23]

Rectal dose is slightly more than oral dose. The child dosage for 15-35 kg is 30 mg twice daily and for over 35 kg is 60 mg twice daily. After rectal dose, plasma concentration plateaus at 20ng/ml and the action lasts for 1-5 hours.

Diclofenac sodium suppository

100mg suppositories are available. The dosage in inflammation and mild to moderate pain, by mouth or by rectum: 6 months to 18 yrs 0.3-1 mg/kg (max 50 mg) 3 times daily. In post-operative pain: 6-18 yrs 0.5-1 mg/kg (max 75 mg) twice daily for maximum 4 days; total daily dose may alternatively be given in 3 divided doses. Absorption from suppository takes 4-5 hours, bioavailability is low (55%) as defecation may remove the drug from the absorption site before complete absorption.[24]

Hyoscine N- butyl bromide (HBB) / Buscopan suppositories

Available strength is 7.5mg. Even though it is used widely in our country, currently there are no studies available on its use for abdominal pain in children.

Conclusion

Equal antipyretic effectiveness has been noted with oral and rectal paracetamol in various studies and hence there is no evidence to support the belief that suppositories whether prescribed in standard dose or at double the dose are superior to oral paracetamol in terms of rapidity of action or in the extent of temperature reduction.

In some parts of the world, rectal administration of drugs is acceptable. Even today world market share for rectal formulations is minimal. In India there are only very few rectal preparations available for children. Most suppositories are not included in the WHO essential medcines list for children (EMLc) or IAP drug formulary. Suppositories are useful when oral administration of drugs is not possible and when the drug needs to act locally.

Points to Remember

- ***Suppositories are not superior to oral medications in terms of rapidity of onset of action.***
- ***It should be considered only in conditions where there is practical difficulty in giving the oral medications or when there is specific indication.***
- ***Many of the rectal formulations which are available elsewhere are not currently available in India.***

References

1. Van Hoogdalem E, de Boer AG, Breimer DD. Pharmacokinetics of rectal drug administration, Part I. General considerations and clinical applications of centrally acting drugs. Clin Pharmacokinet 1991;21: 11-26.
2. Gross HM, Becker CH. A Study of Suppository Bases. J Pharm Sci 1953;42:90-95.
3. Polishchuk AY, Efremovich G. Application of Polymer systems. Multicomponent Transport in Polymer Systems for Controlled Release; 3:195.
4. Jannin V, Lemagnen G, Gueroult P, Larrouture D, Tuleu C. Rectal route in the 21st Century to treat children. Adv Drug Deliv Rev 2014;73: 34–49.
5. Joint formulary committee. British National Formulary for Children. BMJ group 2015; 69.
6. Scolnik D, Kozer E, Jacobson S, Diamond S, Young NL. Comparison of oral versus normal and high-dose rectal paracetamol in the treatment of febrile children. 2002;110(3):553-556.
7. Nabuls M, Tamim H, Sabra R, Mahfoud Z, Malaeb S, Fakih H, et al. Equal antipyretic effectiveness of oral and rectal paracetamol : a randomized controlled trial. BMC Pediatrics 2005;5:35.
8. Owczarzak V, Haddad J Jr. Comparison of oral versus rectal administration of paracetamol with codeine in postoperative pediatricadenotonsillectomy patients. Laryngoscope. 2006;116(8):1485-1488.
9. Knudsen FU. Plasma-diazepam in infants after rectal administration in solution and by suppository. Acta Pediatr Scand 1977;66(5):563-567.
10. Dhillon S, Ngwane E, Richens A. Rectal absorption of diazepam in epileptic children. Arch Dis Child 1982;57:264-267.
11. Hirabayashi Y, Okumura A, Kondo T, Magota M, Kawabe S, Kando N,et al. Efficacy of a diazepam suppository at preventing febrile seizure recurrence during a single febrile illness. Brain Dev 2009 ;31:414-418.
12. Chiang LM, Wang HS, Shen HH, Deng ST, Tseng CH, Chen YI, et al. Rectal diazepam solution is as good as rectal administration of intravenous diazepam in the first-aid cessation of seizures in children with intractable epilepsy. Pediatr Neonatol 2011;52:30-33.
13. Godbole PP, Pinfield A, Stringer MD. Idiopathic megarectum in children. Eur J Pediatr Surg 2001;11: 48-51.
14. Burgers R, Bonanno E, Madarena E. The care of constipated children in primary care in different countries. ActaPaediatr 2012;101:677-680.
15. Portalatin M, Winstead N. Medical Management of Constipation. Clin Colon Rectal Surg. 2012;25:12–19
16. Heyman MB, Kierkus J, Spénard J. Efficacy and safety of mesalamine suppositories for treatment of ulcerative proctitis in children and adolescents. Inflamm Bowel Dis 2010;16:1931-1939.
17. Gionchetti P, Rizzello F, Venturi A. Comparison of oral with rectal mesalazine in the treatment of ulcerative proctitis. Dis Colon Rectum 1998;41:93-97.
18. Allgayer H, Kruis W, Eisenburg J, Paumgartner G. Comparative pharmacokinetics of sulphasalazine and sulphapyridine after rectal and oral administration to patients with ulcerative colitis.Eur J Clin Pharmacol 1984;26:275-277.
19. Gomes MF, Faiz MA, Gyapong JO. Pre-referral rectal artesunate to prevent death and disability in severe malaria: a placebo-controlled trial. Lancet 2009;373(9663): 557-566.
20. Gomes M, Ribeiro I, Warsame M, Karunajeewa H, Petzold M. Rectal artemisinins for malaria: a review of efficacy and safety from individual patient data in clinical studies. BMC Infect Dis 2008; 8:39.

21. Arvidsson J, Nilsson HL, Sandstedt P. Replacing carbamazepine slow-release tablets with carbamazepine suppositories: a pharmacokinetic and clinical study in children with epilepsy. J Child Neurol 1995;10:114-117.
22. Matsumoto Y, Watanabe Y, Yamamoto I. Difference in rectal absorption of morphine from hollow-type and conventional suppositories in rabbits. Biol Pharm Bull 1993; 16(2):150-153.
23. Lencz L. The importance of domperidone (Motilium) in controlling postoperative nausea and vomiting. Ther Hung 1990;38:106-109.
24. Amidona, Smitha DE. Determination of the Population Pharmacokinetic Parameters of Sustained-Release and Enteric-Coated Oral Formulations, and the Suppository Formulation of Diclofenac Sodium by Simultaneous Data Fitting Using NONMEM., Biopharm. Drug Dispos 1998;19:169–174.

NEWS AND NOTES

22ND ANNUAL CONVENTION OF TWIN CITIES BRANCH of IAP
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DERMATOLOGY

CUTANEOUS ADVERSE DRUG REACTIONS

Madhu R

Abstract: *Cutaneous adverse drug reactions (CADR) form a spectrum ranging from benign conditions to serious life-threatening reactions such as Steven Johnson syndrome, toxic epidermal necrolysis and drug hypersensitivity syndromes. In the latter conditions, skin manifestations do not occur in isolation and present as a systemic reaction. Various mechanisms, both immunological and non-immunological have been postulated to explain CADR. Antimicrobials, nonsteroidal anti-inflammatory drugs and anticonvulsants are the most common agents implicated in CADR. Exanthematous type which is the most common of CADR, often poses a diagnostic dilemma due to its close resemblance to viral exanthems. The most important step in the management is to withhold the offending agent and all the non-essential drugs. Early diagnosis and prompt treatment of severe cutaneous drug reactions pave the way to reduce the morbidity and improve the quality of life of these children.*

Keywords: *Cutaneous adverse drug reaction, Sulfonamides, Anticonvulsants, Steven Johnson syndrome, Toxic epidermal necrolysis.*

In day-to-day clinical practice, many instances of adverse drug reactions are seen which most of the time are benign but occasionally are life threatening. Polypharmacy and immunosuppression have increased the incidence of drug reactions. World Health Organisation (WHO) defines 'adverse drug reaction' as 'a response to a medicine which is noxious and unintended and which occurs at doses normally used in human for the prophylaxis, diagnosis or therapy of disease, or for the modification of physiological function'.[1] Cutaneous adverse drug reactions (CADR) was reported in 35% of all adverse drug reactions (ADR) reports submitted to WHO ADR VigiBase.[2] There is limited data available with regard to the epidemiology of the cutaneous adverse drug reactions in children in India. Western literature states that CADR are the most common adverse drug reactions seen in hospitalized children with an incidence of 2%-3%.[3] Studies conducted among the out patients have shown that 2.5% of children treated with a drug may develop CADR. This figure may increase to up to 12% if an antibiotic was given.[4]

Various factors like age of the patient, sex, genetic predisposition, associated diseases, impaired renal and liver function, duration of hospital stay and drug related factors such as dosage, duration of treatment, type of drug, route of administration and polypharmacy have an impact on the incidence and severity of adverse drug reactions.[5] Drugs with a tendency to produce reactive intermediates or toxins, low therapeutic indices and high levels of drug interactions are more prone to result in drug reactions. It has been found that boys younger than 3 years and girls older than 9 years are more vulnerable to develop adverse drug reactions.[6]

Classification

ADR may be simply classified into immediate reactions that occur within 1 hour of administration of the drug and the delayed type which includes reactions that occur after 1 hour, usually beyond 6 hours and sometimes after days or even weeks. According to Rawlins and Thompson's classification based on dose relation and predictability, adverse drug reactions are grouped into 2 types namely type A and type B. Type A reactions which are predictable and dose dependent, occur due to an exaggerated, but normal pharmacological action of a drug in therapeutic dosage. Type B reactions result from an idiosyncratic or bizarre response that is unexpected from the known pharmacological response of a drug given in therapeutic dose. They are dose independent, unpredictable, severe reactions associated with high morbidity and mortality.[7]

Dose, time and susceptibility (DoTS) classification which provides a complete evaluation of the ADR is ideal for pharmacovigilance studies and is superior to that of Rawlins. Based on the dose, ADR are classified as supratherapeutic (higher than recommended dose), collateral (recommended dose) and hypersensitive (lower dose). With regard to the temporal relationship, they are

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classified as time-independent or time-dependent reactions. Time-dependent reactions are further classified as fast, first dose, early, intermediate, late and delayed reactions. Various factors such as genetic susceptibility, age, gender, presence of exogenous factors and disease are taken into consideration with regard to the susceptibility.[8]

Pathogenesis[6,9]

Cutaneous drug eruptions are mediated by immunological or non-immunological mechanisms. Most of the adverse drug reactions (almost 75%-80%) are due to predictable, non-immunologic factors. Those that are caused by unpredictable effects account for 20%-25% and may be either immune mediated or non-immune mediated. Sometimes, it may be difficult to clearly point out the underlying mechanism in certain drug eruptions. Immunologically mediated drug eruptions which constitute 5%-10% of all drug reactions are unpredictable and may induce cell mediated immunity or humoral immunity. Gell-Coomb's Modified classification of drug hypersensitivity is given in Table I.[10]

Specific T cell activation is said to be responsible for morbilliform rash due to sulfonamide. Fas / Fas ligand (FasL) induced apoptosis plays an important role in Steven Johnson syndrome **(**SJS) and toxic epidermal necrolysis (TEN). Apart from the immune basis, genetic predisposition has also been stated to contribute to the pathophysiology in conditions such as 'drug rash with eosinophilia and systemic symptoms' (DRESS) and 'acute generalized exanthematous pustulosis' (AGEP).

Predictable factors responsible for non-immunologically mediated drug reactions include overdose, pharmacologic side effects, cumulative toxicity, delayed toxicity, drug-drug interactions, alterations in metabolism and exacerbation of pre-existing disease. Unpredictable factors are intolerance and idiosyncragy.

Approach to drug eruptions

History: Detailed history of all medications including over the counter and alternative drugs used over a period of 8-12 weeks is mandatory while trying to find the offending agent. When multiple drugs are given, it would become a difficult task to delineate the agent responsible for the skin reaction. Most of the drug eruptions occur over a period of 1-6weeks after consumption of the drug. But drug induced lupus may take up to 3 years of therapy to manifest.[11] If a person has been sensitized to a drug, then the drug rash may occur within a week. Drug history should include the date of starting the drug, route, duration, dosage, frequency and the time gap between starting the drug and the onset of lesions. One should ask for previous history of adverse reaction to drug or food, type of reaction, how soon the drug was withdrawn, improvement after cessation of the drug and recurrence on re-exposure to the drug. History of concomitant infections that may cause drug eruption or act as co-factors (as in infectious mononucleosis and ampicillin induced rash or HIV and trimethoprim sulfamethoxazole drug reaction) should be taken. History should be taken also with regard to vaccination, metabolic disorders and immune suppression. Personal or family history should be sought with regard to any skin disease or

Table I. Gell-Coomb's modified classification of drug hypersensitivity

Reaction type	Mediator	Example
Type I	IgE	Drug induced urticaria, angioedema, anaphylaxis
Type II	IgG and Fc	Drug induced thrombocytopenia (heparin),hemolytic anemia (penicillin)
Type III	IgG and complement or Fc receptor	Drug induced vasculitis (Minocycline), SLE (quinidine), certain types of urticaria, serum sickness
Type IVa	Monocyte mediated (IFN-*y*)	Maculopapular rash, contact dermatitis
Type IVb	Eosinophil mediated (IL-5 and IL-4)	Maculopapular rash with eosinophilia
Type IVc	T-cell (perforin and granzyme B)	SJS/TEN
Type IVd	Neutrophil mediated (IL-8)	Acute generalized exanthematous pustulosis

Table II. Naranjo ADR probability scale

Questions	Yes	No	Don't know
Are there previous conclusive reports on this reaction?	+1	0	0
Did the adverse event appear after the suspected drug was administered?	+ 2	-1	0
Did the adverse reaction improve when the drug wasdiscontinued or a specific antagonist was administered?	+1	0	0
Did the adverse reaction reappear when the drug wasreadministered?	+2	-1	0
Are there alternative causes (other than the drug) thatcould on their own have caused the reaction?	-1	+2	0
Did the reaction reappear when a placebo was given?	-1	+1	0
Was the drug detected in the blood (or other fluids) in aconcentration known to be toxic?	+1	0	0
Was the reaction more severe when the dose was increased.or less severe when the dose was decreased?	+1	0	0
Did the patient have a similar reaction to the same orsimilar drugs in any previous exposure?	+1	0	0
Was the adverse event confirmed by any objectiveevidence'?	+1	0	0

Scoring for Naranjo scale: > 9 – Definite ADR; 5-8 – Probable ADR; 1-4 – Possible ADR; 0- Doubtful ADR

hypersensitivity syndromes.[6,9] Sometimes, it becomes very difficult to find the offending drug that has caused the adverse reaction. Naranjo ADR probability scale is a widely used causality.

Clinical spectrum

Morphology of cutaneous adverse reactions may range from a classical drug reaction pattern or may mimic other skin conditions. Various benign presentation of CADR are exanthematous, urticarial, bullous, lichenoid, acneiform, fixed drug eruption and photosensitive reactions. Severe forms include SJS / TEN, drug induced hypersensitivity syndrome (DHS) and acute generalized exanthematous pustulosis (AGEP). While exanthematous drug eruption has been found to be the most common type, studies from Singapore and Turkey have reported urticaria and angioedema to be the most common type of CADR.[14,15]

Exanthematous drug eruptions

Exanthematous eruption also called as morbilliform drug eruption accounts for 95% of skin reactions and is thus the most common form of CADR seen in children. Erythematous maculopapular rash which usually starts on the trunk is seen during the first 5-14 days of treatment. Lesions may be scarlatiniform, morbilliform or rubelliform and are associated with pruritus. During resolution that occurs within 7-14 days, erythema fades to be followed by desquamation. Exanthematous eruption is caused by many drugs, most common being penicillins, sulphonamides, aromatic anticonvulsants, non-steroidal anti-inflammatory drugs (NSAIDs), non-nucleoside reverse transcriptase inhibitors (e.g. nevirapine). Drug-viral interaction results in an exanthematous rash e.g. patients with infectious mononucleosis treated with ampicillin and patients with HIV treated with sulphonamides. Sometimes, it may become difficult to distinguish exanthematous drug eruption from viral exanthems, especially when a child develops a rash after starting treatment for fever. Viral exanthem starts on the face and progresses to the trunk. Pruritis and involvement of palms and soles is seen in drug eruptions.[4,5,6,11]

Drug induced urticaria

Drug induced urticarial wheals may occur alone, which is more common or may present along with angioedema and anaphylaxis. Wheals may occur within 24-36 hours after consumption of the drug. However, when re-challenge is done, lesions develop within minutes.

Penicillins, aspirin, NSAIDs, sulphonamides, opiates, radiocontrast media are the common drugs implicated in causing urticarial lesions.[11,16]

Fixed drug eruption (FDE)

FDE is seen more often in adults than in children. Study done by Sharma et al in 50 children and adolescents in North India reported FDE in 22% of children, second next to maculopapular rash (26%).[17] FDE is characterized by the occurrence of pruritic, well circumscribed, erythematous, edematous plaques at the same sites, whenever the particular offending drug is taken. Lesions are violaceous or greyish blue in colour and become hyperpigmented later. Common sites involved are lips, trunk, arms, legs and genitals. Lesions may be solitary or few in number. Generalised FDE in children due to trimethoprim sulphamethoxazole and nimesulide have been reported.[18,19] Rarely systemic symptoms may be present. If re-challenge is done, lesions recur at the existing sites while new lesions may also occur within 30minutes to 8hours. Commonly associated drugs are sulphonamides, barbiturates, NSAIDs, antihistamines (diphenhydramine, hydroxyzine, loratadine), anticonvulsants, tetracyclines, salicylates, metronidazole, rifampicin, penicillins, erythromycin and phenolphthalein. Though FDE is self-limiting, residual hyperpigmentation persists for many months.[11,16,20,21]

Acneiform eruptions

Acneiform eruptions are characterized by sudden, abrupt onset of monomorphic papules and pustules with comedones being markedly absent. Non-seborrheic areas such as legs and arms may be involved. Interval between starting the drug and onset of lesions will depend upon the drug, for eg steroid acne may occur within 2 weeks after starting treatment. Common drugs known to cause acneiform eruptions are corticosteroids, anabolic steroids, iodides, bromides, phenytoin, valproate, isoniazid, rifampicin, ethionamide and cyclosporin. Lesions gradually disappear after the cessation of the drug.[11,16]

Severe cutaneous adverse drug reactions

Steven-Johnson syndrome/ Toxic epidermal necrolysis (SJS/TEN)

SJS and TEN, severe cutaneous adverse reactions with an incidence of 1-2 million /year are known for the high morbidity and mortality they cause, in both adults and children.[22] SJS was described in 1922 in 2 children, to be followed much later by the description of TEN by Lyell in 1956.[23,24] Clinical spectrum of these bullous eruptions is divided into 3 categories based on the degree of epidermal detachment:[25]

(1) SJS is epidermal detachment of <10% of the total body surface area (BSA)

(2) SJS / TEN overlap is detachment between 10% - 30% BSA

(3) TEN is detachment > 30% of BSA with widespread macules or flat atypical targets

While TEN is almost always caused by drugs, SJS may sometimes be caused by infections especially Mycoplasma pneumoniae. Human herpes virus 6 and7, Epstein Barr virus, cytomegalo virus, parvo virus, group A beta-hemolytic streptococci, mycobacterium, rickettsia, auto immune disorders, neoplasia and vaccination may act as potential cofactors or triggers along with the drugs.[22] Sulfonamides, penicillins, cephalosporins, quinolones, phenobarbitone, phenytoin, carbamazepine, lamotrigine, NSAIDs are the common drugs that have been found to trigger SJS/TEN.[11,25,26] Study done in Han Chinese population has shown that patients carrying HLA-B*1502 are at increased risk to develop carbamazepine induced SJS/TEN.[27] The time gap between starting the drug and onset of SJS/TEN is shorter with antibiotics in contrast to that of anticonvulsants and allopurinol which are tolerated for many weeks.[28]

Clinical features

Children with SJS/TEN initially present with prodromal symptoms of fever, malaise, headache, coryza, cough, sore throat, myalgia, arthralgia, vomiting and diarrhea, 1-14 days before the onset of skin lesions. Lymphadenopathy and hepatosplenomegaly may be present. In SJS, skin lesions initially begin as erythematous or purpuric macules and progress to blister formation and epidermal detachment limited to 10% BSA. Atypical target lesions may be present. Face and trunk are the most affected and the lesions spread centrifugally. Mucosal involvement may occur 1-2days prior to the onset of skin lesions with at least 2 mucosae being involved namely the eyes and oral cavity. Hemorrhagic crusts over the lips and mucosal erosions and ulceration will be present. In patients with TEN, erythema is widespread and confluent with bullae resulting in extensive denudation of the skin. Tenderness of the skin is the striking feature in the early phase. Nikolsky sign is positive i.e on application of gentle tangential pressure on an area of erythema, skin in the patients with SJS/TEN peels off. There is extensive mucosal involvement of conjunctiva, oral mucosa, anogenital mucosa, trachea, bronchi and oesophagus.

Ocular manifestations include photophobia, purulent conjunctivitis, corneal ulceration, keratitis, uveitis and panophthalmitis.[29] Ophthalmic sequelae is seen in 40% of patients and may be catastrophic at times resulting in partial or complete blindness. Complications include thermal dysregulation, electrolyte imbalance and multiple organ involvement with secondary septicemia. Almost 45% of children may be left with long term sequelae in the form of dyschromia and persistent nail dystrophy.[14,25]

Drug reaction with eosinophilia and systemic signs (DRESS)

DRESS also known as drug induced hypersensitivity syndrome (DHS) is characterized by the presence of exanthematous rash with fever, swelling of face (especially periorbital), pruritus, conjunctivitis, arthralgia and systemic involvement that may include hepatic, renal, central nervous system, cardiac and pulmonary systems. Other systemic manifestations that may present are agranulocytosis, hypogammaglobulinemia, pancreatitis, epididymitis, myositis and colitis. Onset is usually seen 1-6 weeks after starting the drug. A study done in Kerala found the latency period to range from 21 days to 90 days.[30] Sulfonamides and aromatic anticonvulsive agents (phenytoin, carbamazepine, phenobarbital) are the most common drugs causing DRESS. Other drugs implicated are lamotrigine, amoxicillin, dapsone, allopurinol, azithromycin and nevirapine. Skin lesions tend to persist for weeks to months after stopping the drug. After an initial period of improvement, there will be a flare of both cutaneous and visceral involvement. Absence of mucosal involvement helps to differentiate DRESS from SJS.

Acute generalized exanthematous pustulosis (AGEP)

AGEP, though more common in adults, has been reported in children. It is characterized by an acute onset of non- follicular sterile pustules on an edematous, erythematous base in association with fever. In addition, target lesions, vasculitis, purpura, blisters, mucosal erosions may be present along with edema of the face. Drugs that are known to trigger AGEP are amoxicillin-clavulanate, cephalosporins, macrolides, clindamycin, terbinafine and contrast agents. Once the offending agent is stopped, there is spontaneous resolution with generalized desquamation within 2 weeks.

Management of CADR

Laboratory investigations are not warranted in the benign or non-febrile forms of CADR. In patients with SJS/TEN or DRESS, complete hemogram, (anemia, leukocytosis, eosinophilia), erythrocyte sedimentation rate (raised), liver function tests (elevated transaminases), renal function tests, serum electrolytes, urine analysis (proteinuria, microscopic haematuria), pus culture and blood culture should be done. Skin biopsy in SJS and TEN will reveal the necrotic keratinocytes. Skin tests like prick test and intradermal tests are useful to confirm IgE mediated immediate hypersensitivity reactions. In case of FDE, patch test done at the site of a previous lesion yields a positive response in 30% patients. In case of over dosage, drug levels could be done.

The first and foremost step in the management of CADR is to find and stop the offending drug and all the non-essential drugs. Exanthematous drug eruption responds well to application of soothing lotions, emollients, topical corticosteroids and oral antihistamines. Second generation antihistamines are preferred in the treatment of urticarial drug eruption. In children with fixed drug eruptions, parents should be educated to avoid the offending drug. Medium potent topical corticosteroids and oral antihistamines will relieve the erythema and pruritus in these children. Topical benzoyl peroxide and tretinoin are useful in the treatment of acneiform eruptions.

Children diagnosed with SJS/TEN should be admitted in the intensive care unit so that close monitoring of thermal regulation, electrolytes, fluid replacement and sepsis prevention could be done. Ophthalmic consultation should be sought and proper care of the eyes should be ensured.

Saline compresses for the crusted areas and antibiotic creams for the erosions are to be applied. Use of intravenous systemic corticosteroids is controversial in TEN. IV immunoglobulins have been found to be of limited use. Recent studies have found cyclosporin to be effective in TEN.[31,32] Treatment of DRESS is similar to that of TEN.

Conclusion

In our country, antibiotics and NSAIDs are easily available over the counter (OTC). In addition to the rampant practice of self-medication, there is ignorance of drug allergy. Hence, it is important to educate every parent against the use of OTC preparations. In the event of CADR, parents should be provided with a drug card with the name of the medication and the list of drugs that are to be avoided. Given the fact that any drug can cause a rash, it is important for us to be well versed with the adverse reactions of various drugs. With regard to severe cutaneous drug reactions, high degree of suspicion, early diagnosis, instant cessation of the offending drug and prompt management in an intensive care unit will definitely improve the clinical outcome and quality of life of the child.

Points to Remember

- ***Cutaneous adverse drug reactions are the most common adverse drug reactions seen in hospitalized children.***
- ***Drugs with a tendency to produce reactive intermediates or toxins, low therapeutic indices and high levels of drug interactions are more prone to result in drug reactions.***
- ***Dose, time and susceptibility (DoTS) classification provides a complete evaluation of the ADR and is ideal for pharmacovigilance studies.***
- ***Detailed history regarding the drug and evolution of the eruption and astute clinical examination will help in correct diagnosis and appropriate management.***

References

1. Dhar S, Banerjee R, Malakar R. Cutaneous drug reactions in children. Indian J Paediatr Dermatol 2014;15:5-11.
2. Aagaard L, Hansen EH. Cutaneous adverse drug reactions in children: a national register based study. Br J Dermatol 2013;168:pp434-437.
3. Pastrana LC, Ghannadan R, Rieder MJ, Dahlke E, Hayden M, Carleton B. Cutaneous adverse drug reactions in children: an analysis of reports from the Canadian pharmacogenomics network for drug safety (CPNDS). J Popul Ther Clin Pharmacol 2011;18:106-120.
4. Shear NH, Knowles SR. Cutaneous reactions to drugs. In: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffel DJ, Wolff K, (Eds). Fitzpatrick's dermatology in general medicine. 8th edn. New York: The McGraw-Hill companies 2012; pp449-457.
5. Ghosh S, Leelavathi D. Acharya, Padma GM Rao. Study and evaluation of the various cutaneous adverse reactions in Kasturba hospital, Manipal. Indian J Pharm Sci 2006; 68:212-215.
6. Nayak S, Acharjya B. Adverse cutaneous drug reactions. Indian J Dermatol 2008;53:2-8.
7. Kanneh AB. Adverse drug reactions in children Part I. Pediatric nursing 2004;16:32-35.
8. Ospina CC, Rojas CB. The DoTS classification is a useful way to classify adverse drug reactions: a preliminary study in hospitalized patients. Int J Pharm Pract 2010;18:230–235.
9. Revuz J, Allanore LV. Drug reactions. In: Bolognia JL, Jorizzo JL, Schaffer JV, (Eds). Dermatology vol 2. 3rd edn. Philadelphia: Elsevier Saunders 2012;pp335-356.
10. Ghosh K, Banerjee G, Ghosal A, Nandi J. Cutaneous Drug Hypersensitivity: Immunological and Genetic Perspective. Indian J Dermatol 2011;56:137-144.
11. Lansang P, Weistein M, Shear N. Drug reactions. In: Schachner LA, Hansen RC, (Eds). Pediatric dermatology, vol.2, 4th edn. Philadelphia: Mosby Elsevier 2011;pp1698-1711.
12. Naranjo CA, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA, et al. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther 1981; 30: 239-245.
13. Syed Ahmed Zaki. Adverse drug reaction and causality assessment scales. Lung India 2011;28:152-153.
14. Dilek N, Özkol HU, Akbaº A, Kýlýnç F, Dilek AR, Saral Y, et al. Cutaneous drug reactions in children: a multicentric study. Postep Dermatol Alergol 2014;31:368-371.
15. Khoo BP, Giam YC. Drug Eruptions in Children: A Review of 111 Cases Seen in a Tertiary Skin Referral Centre. Singapore Med J 2000;41:525-529.
16. Jones MRA, Lee HY. Benign cutaneous adverse reactions to drugs. In: Griffiths CEM, Barker J, Bleiker T, Chalmers R, Creamer D. Editors. Rook's Textbook of dermatology. 9th edn. West Sussex: Wiley Blackwell, 2016p118. 1-118.17.
17. Sharma VK, Dhar S. Clinical pattern of cutaneous drug eruption among children and adolescents in north India. Pediatr Dermatol 1995;12:178-183.
18. Can C, Akkelle E, Bay B, Arýcan O, Yalcin O, Yazicioglu M. Generalized fixed drug eruption in a child dueto trimethoprim/sulfamethoxazole. Pediatr Allergy Immunol 2014;25:413–415.
19. Sarkar R, Kaur C, Kanwar AJ. Extensive fixed drug eruption to Nimesulide with cross-sensitivity to sulphonamides in a child. Pediatr Dermatol 2002;19:553-554.
20. Heelan K, Shear NH. Cutaneous Drug Reactions in Children: An Update. Pediatr Drugs 2013;15:493-503.
21. Song JE, Sidbury R. An update on pediatric cutaneous drug eruptions. Clin Dermatol 2014; 32:516–523.
22. Pulido CF, Patos VG. A review of causes of Steven-Johnson syndrome and toxic epidermal necrolysis in children. Arch Dis Child 2013;98:998–1003.
23. Steven AM, Johnson FC. A new eruptive fever associates with stomatitis and ophthalmia; report of two cases in children. Am J Dis Child 1922;24:526–533.
24. Lyell A. Toxic epidermal necrolysis: an eruption resembling scalding of the skin. Br J Dermatol 1956;68:355-361.
25. The hypersensitivity syndromes. In: Paller AS, Mancini AJ (Eds). Hurwitz clinical pediatric dermatology. 4th edn. Philadelphia: Elsevier Saunders 2011; pp467-494.
26. Sethuraman G, Sharma VK, Pahwa P, Khetan P. Causative drugs and clinical outcome in Steven Johnson Syndrome (SJS), Toxic Epidermal Necrolysis (TEN) and SJS-TEN overlap in children. Indian J Dermatol 2012;57:199-200.
27. Koh MJ, Tay YK. Steven-Johnson syndrome and toxic epidermalnecrolysis in Asian children. J Am Acad Dermatol 2010;62:54-60.

28. Markovi MA, Medjo B, Jankulovi MG, Velickovic TC, Nikolic D, Nestorovic B. Steven-Johnson syndrome and toxic epidermal necrolysis in children. Pediatr Allergy Immunol 2013;24:645–649.

29. Sasidharanpillai S, Riyaz N, Khader A, Rajan U, Binitha MP, Sureshan DN. Severe cutaneous adverse drug reactions: A clinic epidemiological study. Indian J Dermatol 2015;60:102.

30. Catt CJ, Hamilton GM, Fish J, Mireskandari, K, Ali A. Ocular Manifestations of Steven-Johnson Syndrome and Toxic Epidermal Necrolysis in Children. J Ophthalmol 2016;166:68-75.

31. Shokeen D. Cyclosporine in SJS/TEN management: a brief review. Cutis 2016; 97: E17-18.

32. Singh GK, Chatterjee M, Verma R. Cyclosporine in Steven-Johnson syndrome and toxic epidermal necrolysis and retrospective comparison with systemic corticosteroid 2013;79:686-692.

CLIPPINGS

A randomized controlled trial evaluating the efficacy of oral sucrose in infants 1 to 3 months old needing intravenous cannulation.

To compare the efficacy of an oral sucrose versus placebo in reducing pain in infants 1 to 3 months of age during intravenous (IV) cannulation in the Emergency Department (ED).

A randomized, double-blind, placebo clinical trial was conducted. Participants were randomly allocated to receive 2 ml of an oral 88% sucrose solution or 2 ml of a placebo solution orally. The outcome measure were mean difference in pain score at one minute post IV cannulation assessed by the Face, Legs, Activity, Cry and Consolability Pain Scale (FLACC) and the Neonatal Infant Pain Scale (NIPS), crying time and variations in heart rate.

Administration of an oral sucrose solution in infants 1 to 3 months of age during IV cannulation did not lead to statistically significant changes in pain scores. However the cry time was significantly reduced.

Desjardins MP, Gaucher N, Curtis S, LeMay S, Lebel D, Gouin S1. A randomized controlled trial evaluating the efficacy of oral sucrose in infants 1 to 3 months old needing intravenous cannulation. Acad Emerg Med. 2016 Apr 21. doi: 10.1111/acem.12991. [Epub ahead of print].

NEWS AND NOTES

HMB & IYCNCON 2016, 6th National Conference of

IYCF Subspecialty Chapter of IAP, Chandigarh, India

Date: 7th – 9th October, 2016

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RADIOLOGY

OSTEOMYELITIS-1

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****Natarajan B**
****Karthik C**
****Arun Prasad S**
****Deebha V**

The clinical diagnosis of osteomyelitis is quite difficult in the early stages, as the presentation can be confusing and results of tests are non-specific. The diagnosis needs to be made quickly and in such a situation, the plain X-ray plays a crucial role in the diagnosis and institution of appropriate therapy to prevent chronic disease.

Osteomyelitis is caused by hematogenous spread of an organism or by direct contamination by injury. The initial site of infection in the bone is the metaphysis where slowing of blood in the looping metaphyseal arteries makes it a favourable site for deposition and multiplication of microbes. In a child less than 18 months of age there are transphyseal arteries that make it possible for infection to extend to the epiphysis and on to the joint. In the X-ray, soft tissue swelling in the muscle plane centred over the joint is enough to make a diagnosis of septic arthritis (Fig.1). In addition joint space can also be widened (Fig.1). Delay in diagnosis and management can lead to destruction of the epiphyses and growth cartilage resulting in shortening of the limb. In Fig.2 the lower femoral epiphysis is completely destroyed and there is lysis extending into the metaphysis.

About two to three days after the bone marrow is infected there is soft tissue swelling in the affected limb. At this point the dilemma is to differentiate between cellulitis (with or without a deep abscess) and osteomyelitis. A plain X-ray can help in the differentiation of these conditions and ultrasound can be useful in locating the abscess. The soft tissue swelling associated with acute osteomyelitis called the 'muscle sign' appears in the muscle plane and is seen circumferentially extending along the whole length of the bone. As the infection continues there is dissolution of the marrow with formation of pus. This is followed by destruction of bone and an attempt to support the weakened bone by formation of periosteal new bone (Fig.3). Some of the dead bone that has separated from the surrounding bone lies trapped within as sequestrum, harbouring the organisms that are inaccessible to antibiotics. Treatment at this stage is far from effective. More new bone forms around the dead weakened sequestrum (Fig.4). This is called involucrum. Fig 4 also shows a long segment of the shaft of the femur sequestered within the dense white involucrum. The extent of destruction can be so severe that an entire bone can lie as sequestrum.

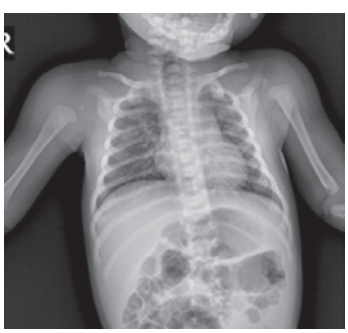

Fig.1.Septic arthritis- right shoulder

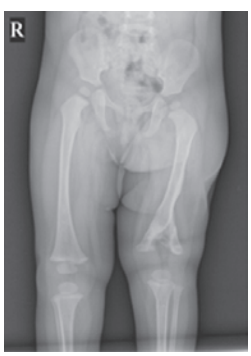

Fig.2.Septic arthritis sequelae - left knee

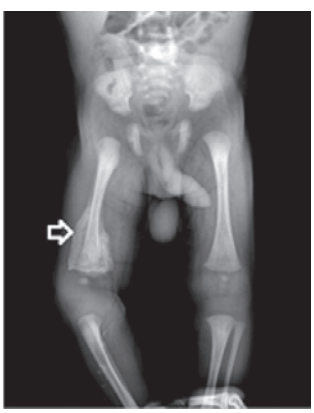

Fig.3.Chronic osteomyelitis - formation of involucrum (arrow)

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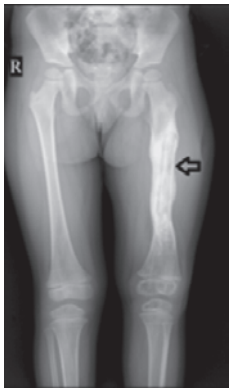

Fig.4.Chronic osteomyelitis - Note long segment of sequestrum / arrow) surrounded by involucrum

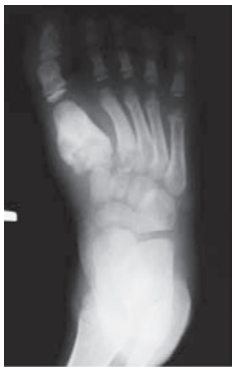

Fig.5.Dactylitis

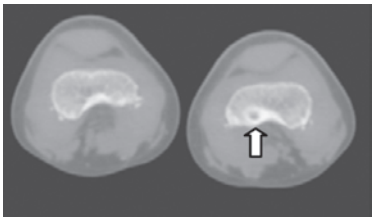

Fig.6. CT Lower femur - Brodie's abscess (arrow)

Dactylitis is osteomyelitis of the short long bones of the hands and feet. The nutrient artery in the long bones enters the diaphysis, divides into two and ascends or descends to both ends of bone. In the short long bones the nutrient artery arborises into a plexus of vessels immediately on entering the diaphyses, so that the initial site of infection is the diaphysis itself. The entire bone becomes thickened and sclerotic with areas of bone destruction (Fig.5). Sickle cell anemia is associated with this type of osteomyelitis that follows infarction of the bone.

Brodie's abscess is a type of osteomyelitis consisting of an abscess in the bone. The patient's immunity is able to fight the virulence of the organism and the pus maybe sterile. Fig.6 is that of a child who came with chronic pain in the knee. The X-ray was not very helpful with faint, ill-defined sclerosis. CT revealed a small lytic lesion in the cortex of the lower femoral epiphysis surrounded by a ring of sclerosis. The differential diagnosis includes osteoid osteoma. However the intensity of the sclerosis around a nidus of an osteoid osteoma will be much more. We will see more about osteomyelitis and cross sectional imaging in the next issue.

CLIPPINGS

Is the QuantiFERON-TB Gold test (QFT) better than the Tuberculin Skin Test (TST) in diagnosing active and latent tuberculosis in BCG-vaccinated children?

This study was done to compare the QuantiFERON–TB Gold test (QFT) and Tuberculin Skin Test (TST) in the diagnosis of active and latent TB in predominantly BCG–vaccinated children. The QFT and TST were only moderately sensitive, but highly specific in ruling out TB and showed good concordance in TB–negative children. Although a case may be made for using both tests in BCG–vaccinated children, the higher costs and technical expertise required for the QFT do not support its use instead of the cheaper and simpler TST in India.

Thomas L, Michael JS, Verghese VP, Chacko A, Jeyaseelan V. Is the QuantiFERON-TB Gold test (QFT) better than the Tuberculin Skin Test (TST) in diagnosing active and latent tuberculosis in BCG-vaccinated children? International Journal of Infectious Diseases 2016;45:342-343.

NEWS AND NOTES

8th Asian Congress of Pediatric Infectious Diseases (ACPID)

Thailand, November 7-10, 2016

Enquiries to:

Email: secretariat@acpid2016.com Website: www.acpid2016.com

CASE REPORT

INTESTINAL STRONGYLOIDIASIS IN AN IMMUNOCOMPETENT BOY

***Sumathi B**
****Nirmala D**
*****Bhaskar Raju B**
******Sunil Kumar KS**

Abstract: *Strongyloides stercoralis is endemic in tropical and sub-tropical regions and is often reported in immunocompromised children. Creeping eruption due to dermal entry of larva, rather than the gastrointestinal route, is a common manifestation in chronic strongyloidiasis. Peripheral eosinophilia is often seen; however, its absence does not rule out the disease. Consecutive stool examinations for larva is diagnostic, but may be negative at times. Small bowel biopsy may help in diagnosis in children with chronic gastrointestinal symptoms. Treatment with ivermectin is rewarding. We report an immunocompetent boy presenting with chronic diarrhea, hypoproteinemia, anemia and cachexia due to intestinal strongyloidiasis, diagnosed by duodenal biopsy.*

Keywords: *Duodenal strongyloidiasis, Hypoproteinemia, Malabsorption, Chronic Diarrhea, Immunocompetent, Children.*

Chronic diarrhea with hypoproteinemia is not uncommon in children. Various causes include allergic colitis and intestinal lymphangiectasia in infants and intestinal tuberculosis, inflammatory bowel disease, gastrointestinal lymphoma and eosinophilic enteritis in older children. Intestinal stongyloidiasis is an important infectious cause in tropical and subtropical countries presenting with chronic diarrhea, malabsorption and protein losing enteropathy in immunocompromised children. The infection is usually asymptomatic in immunocompetent individuals. We report an immunocompetent boy who presented with chronic diarrhea and hypoproteinemia due to intestinal strongyloidiasis.

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Case Report

A 12 year old boy from Andhra Pradesh presented with recurrent attacks of watery stools without blood, intermittent low grade fever, anorexia, weight loss for two years, facial puffiness and pedal edema of two months duration. There was no history of wheeze, rash or prolonged drug intake. Clinically he was emaciated, anemic, with facial puffiness and pedal edema. His weight was 24 kg (<5th centile), height 145 cm (between 25th to 50th centile) as per WHO growth chart. The possibilities of intestinal tuberculosis, inflammatory bowel disease, intestinal lymphoma and eosinophilic enteritis were thought of and investigated. CBC showed hemoglobin of 10.2 gms/dL, total leucocyte count 9400 cells/cmm, polymorph 72%, lymphocyte 28%, platelet count 2.8L, ESR 36mm/hour and peripheral smear showed hypochromic microcytic anemia without eosinophilia. Renal and liver function tests were normal. Serum albumin was 1.8 g/dL. Mantoux and HIV Elisa were negative. Serum immunoglobulin and flow cytometry for CD3, CD4, CD19 and CD20 were within normal limits. Stool examination for ova, cyst, larvae and occult blood were negative on three consecutive days. X-ray chest was normal, USG abdomen showed ascites and was tapped. Ascitic fluid revealed protein of 780mg/dL, total cell count <300/microlitre and adenosine deaminase was 12IU/L.

Barium meal follow through showed features of malabsorption. Endoscopy showed normal mucosa of upper gastrointestinal tract and duodenal biopsy was done. Colonoscopy showed normal colonic mucosa up to terminal ileum. Multiple biopsies were taken. Colonic biopsy was not contributory. Duodenal biopsy showed marked villous blunting with focal intra epithelial cytoplasmic large inclusions suggestive of larval form of parasite with inflammatory cell infiltrate in lamina propria without increase in intraepithelial lymphocytes or presence of granuloma (Fig.1).

The boy was treated with tablet ivermectin, two doses at a dose of 200µg/kg orally on day 1 and day 14 along

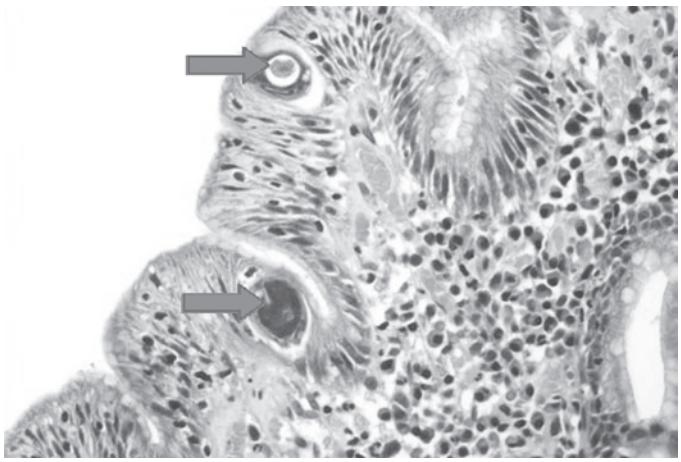

Fig.1. High-power view of a duodenal biopsy showing intraepithelial organisms (larvae) along with an inflammatory infiltrate in the lamina propria

with nutritional rehabilitation. There was dramatic clinical response with subsidence of edema, control of diarrhea with sense of well being and weight gain. He is on follow up for more than a year without any recurrence of symptoms.

Discussion

Strongyloides stercoralis is a soil-transmitted helminthic infection mostly reported in tropical and subtropical countries and with low endemicity in temperate zone.[1] Filariform larva in the soil penetrate intact skin of human beings. The organisms multiply asexually in humans leading to chronic infection. The clinical presentation depends on interaction between host immune response and the agent and varies from non-apparent /asymptomatic infection in an immunocompetent individual to multisystem chronic or even fatal disease in immunosuppressed children, with high mortality rates. Gastrointestinal symptoms include abdominal bloat, chronic diarrhea, abdominal pain, anemia, cachexia, weight loss mimicking conditions like intestinal tuberculosis, inflammatory bowel disease, gastrointestinal lymphoma and eosinophilic enteritis. Peripheral eosinophilia is a common finding. Stool examination for larva is diagnostic but at times may be negative requiring multiple stool tests, as stool output of parasite is low and irregular especially in chronic strongyloidiasis[2,3] Often small bowel biopsy is helpful in such situations. In those with multisystem involvement, larvae can be identified in peritoneal fluid, pleural fluid, lymph nodes, urine specimens and cerebrospinal fluid.[4] Though drugs like albendazole and thiabendazole may be used, the drug of choice is ivermectin at a dose of 200 μg/kg as two doses at 14 days apart. Ivermectin has a cure rate of 94% - 100%[5,6] and is recommended in treatment of intestinal strongyloidiasis. In those countries with high endemicity, simple preventive measures such as improved living conditions, personal hygiene, regular deworming and use of protective footwear are likely to reduce the disease occurence.[7]

Conclusion

Intestinal strongyloidiasis should be considered even in immunocompetent children presenting with chronic diarrhea and hypoproteinemia in tropical countries. Absence of peripheral eosinophilia and negative stool test do not rule out the disease and intestinal biopsy may help in diagnosis. Dramatic response is seen to drugs like ivermectin.

References

1. Olsen A, Van Lieshout L, Marti H, Polderman T, Polman K, Steinmann P, et al. Strongyloidiasis–The most neglected of the neglected tropical diseases? Trans R Soc Trop Med Hyg 2009;103(10):967-972.
2. Miller MA, Church LW, Salgado CD. Strongyloides Hyperinfection: A treatment Dilemma. Am J Med Sci 2008;36:358–361.
3. Siddiqui AA, Berk SL. Diagnosis of Strongyloides stercoralis Infection. Clin Infect Dis 2001;33(7):1041-1047.
4. Chacín-Bonilla L Systemic strongyloidiasis. Review. Invest Clin 1991;32(3):131-145.
5. Marcos LA, Terashima A, Salmavides S, Alvarez H, Lindo F, Tello R, et al. Thiabendazole for the control of Strongyloides stercoralis infection in hyper-epidemic area in Peru Rev Gastroenterol Peru 2005;25:341–348.
6. Mirdha B. Human strongyloidiasis: often brushed under the carpet. Trop Gastroenterol 2009;30(1):1–4.
7. Iriemenam N, Sanyaolu A, Oyibo W, Fagbenro-Beyioku A. Strongyloides stercoralis and the immune response. Parasitol Int 2010;59(1):9–14.

NEWS AND NOTES

12th Asian Society for Pediatric Research

(ASPR) & Faculty of Medicine Ramathibodi Hospital Joint Meeting 2016

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CASE REPORT

TRACHEOMALACIA DUE TO VASCULAR ANOMALY IN A YOUNG CHILD

***Bhavik Langanecha**
***Sumant Prabhudesai**
****Bala Ramachandran**
*****Balakrishnan KR**

Abstract: *Tracheomalacia persisting beyond infancy is uncommon. We report a 3-year-old boy with persistent stridor who required endotracheal intubation and mechanical ventilation due to severe airway obstruction. He was found to have severe tracheomalacia. Imaging showed an anomalous innominate artery causing tracheal compression, which was relieved after aortopexy. Tracheomalacia is common in infancy, but its persistence through early childhood should prompt further investigation to rule out correctable secondary causes.*

Keywords: *Tracheomalacia, Anomalous innominate artery, Aortopexy*

Tracheomalacia is a common cause of stridor in infants. However, it rarely persists beyond infancy. Persistent symptoms in older children need to be investigated for secondary causes. We report the case of a pre-school child with tracheomalacia secondary to an intrathoracic vascular anomaly.

A three-year-old well thriving male child was brought to the emergency room with a short history of cough, fever and fast and noisy breathing. He had a history of noisy breathing in the past, with repeated episodes of respiratory tract infection for which he had required admission at least twice. On examination he was found to have severe respiratory distress with stridor, suprasternal and sternal in-drawing, suggestive of upper airway obstruction. He was treated with humidified oxygen, nebulized bronchodilators and intravenous steroids, but showed no improvement.

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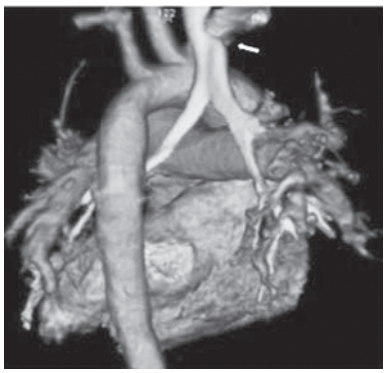

Fig.1. CT pulmonary angiography (reconstruction, posterior view) showing tracheal narrowing due to compression from the innominate artery (marked by arrow).

He was intubated and ventilated for respiratory failure. Once stabilized, flexible laryngoscopy was performed and showed severe tracheomalacia in the middle one-third of the trachea. As this was unusual for this age, a CT thorax with angiography was performed and showed a short segmental narrowing of the trachea at the second and third thoracic vertebral level (D2-D3) due to external compression by an aberrant innominate artery. Echocardiography showed right subclavian artery dilatation at the origin and bifurcation though there were no structural cardiac anomalies. The child underwent an aortopexy in view of symptomatic tracheal compression. Significant relief of compression was noted during intraoperative bronchoscopy. He was extubated successfully, post-surgery.

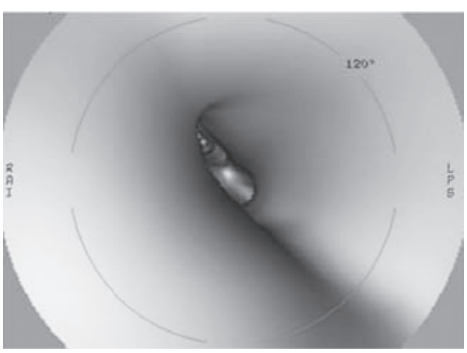

Fig.2. Preoperative virtual bronchoscopy showing tracheal compression from the right side

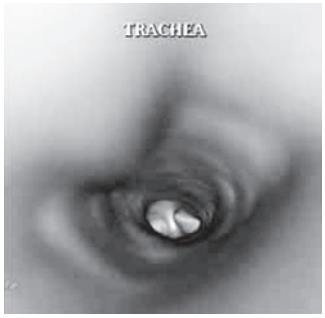

Fig.3. Immediatepostoperative virtual bronchoscopy showing persistence of tracheal narrowing

On follow up, the child is doing well, with no stridor, breathing difficulty or pulmonary infections. Immediately after surgery, virtual bronchoscopy revealed persistence of tracheal lumen compression, but the child was symptomatically much better. No tracheal compression was seen on repeat CT angiography performed 3 months after surgery.

Discussion

Abnormalities of the great vessels of the mediastinum are estimated to occur in approximately 3% of the general population.[1] Most instances of vascular compression of the trachea are asymptomatic and incidental discovery is not uncommon.[2] Children who do develop symptoms are generally diagnosed by one year of age.[1] Of the mediastinal vascular anomalies, Tracheal Compression by Innominate Artery (TCIA) is commonly reported and is a potentially dangerous syndrome leading to airway obstruction.[3, 4, 5] Symptomatic infants and young children characteristically present with expiratory stridor, cough, recurrent bronchopulmonary infections, and occasionally apnea.[1]

Though vascular anomalies may not always contribute to tracheomalacia, about 16% of children with tracheomalacia may have associated TCIA. In a large review on children who underwent aortopexy for tracheomalacia, Torre et al found that only 9% of children had idiopathic tracheomalacia while 87% had relevant structural anomalies like esophageal atresia, vascular rings or TCIA.[6] Children with these associated anomalies were found to have good results after aortopexy. A significant number of cases may have an associated congenital heart disease.[7] A majority of patients with symptomatic TCIA improve spontaneously with age and can be managed conservatively with humidified oxygen, steroids, and antibiotics, whenever required.[8] Surgical intervention may be needed infrequently, if the child has apnea, multiple episodes of tracheobronchitis or bronchopneumonia, or fails to respond to medical therapy.[2,3] Incidental detection is not considered an indication for surgery.[9] No definite protocol has yet been established and no consensus exists regarding preference of procedure.[2,10] Arteriopexy and proximal reimplantation of the innominate artery remain preferred surgical methods.[11]

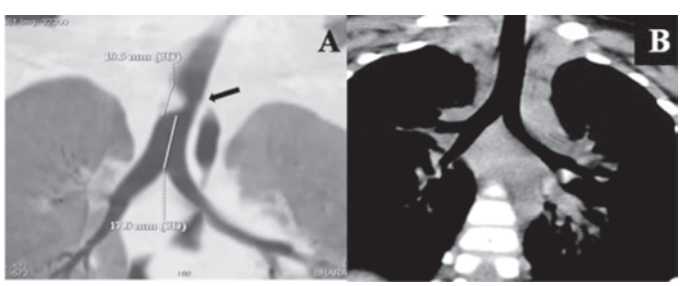

Fig.4.(A): CT thorax showing tracheal narrowing. (B): Post-operative CT thorax with normal tracheal lumen after 3 months

Isolated tracheomalacia often presents as stridor in early infancy and is diagnosed by dynamic airway compression seen on flexible bronchoscopy. In most cases, symptoms resolve when the airways enlarge as the tracheal cartilages grow. Persistence beyond 3 years is rare and should prompt the search for an alternative cause of stridor such as an intrinsic or extrinsic airway compression. This may need a repeat bronchoscopy, CT chest with pulmonary angiography and less frequently, an esophagography.

Conclusion

Tracheomalacia is common in infancy, but its persistence through early childhood should prompt further investigation to rule out correctable secondary causes. Surgical correction of a vascular anomaly may help relieve the tracheal compression.

Points to Remember

- ***Persistence of tracheomalacia beyond infancy is uncommon.***
- ***Such persistence warrants investigation for a correctable cause.***

References

1. Wiatrak BJ. Congenital anomalies of the larynx and trachea. Otolaryngol Clin North Am 2000; 33: 91-110.
2. Sariaydin M, Findik S, Atici AG, Ozkaya S, Uluisik A. Asymptomatic double aortic arch. Int Med Case Rep J 2010; 3: 63–66.
3. Friedman E, Kennedy A, Neitzschman HR. Innominate Artery Compression of the Trachea: An Unusual Cause of Apnea in a 12-Year-Old Boy. South Med J 2003; 96(11): 1161-1164.

4. Mustard WT, Bayliss CE, Fearon B, Pelton D, Trusler GA. Tracheal compression by the innominate artery in children. Ann Thorac Surg 1969; 8:312-319.
5. Gross RE, Neuhauser EBD. Compression of the trachea by an anomalous innominate artery: An operation for its relief. Am J Dis Child 1948; 75: 570-574.
6. Torre M, Carlucci M, Speggiorin S, Elliott MJ. Aortopexy for the treatment of tracheomalacia in children: review of the literature. Ital J Pediatr 2012; 38: 62-70.
7. Sachdev MS, Joshi R, Kaul S, Kohli V. Innominate Artery Compression of Trachea. Indian J Pediatr 2007; 74 (8): 768- 769.
8. Strife JL, Baumel AS, Dunbar JS. Tracheal compression by the innominate artery in infancy and childhood. Radiology 1981; 139: 73-75.
9. Myer CM, Wiatrak BJ, Cotton RT. Innominate artery compression of the trachea: Current concepts. Laryngoscope 1989; 99: 1030-1034.
10. Minagawa T, Oizumi H, Emura T, Sadahiro M .Tracheal stenosis treated by division of the brachiocephalic artery: Report of a case. Surg Today 2010 Dec; 40(12):1152-1154.
11. Hawkins JA, Bailey WW, Clark SM. Innominate artery compression of the trachea: Treatment by reimplantation of the innominate artery. J Thorac Cardiovasc Surg 1992; 103: 678-682.

BOOK REVIEW

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