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GENOTYPE-PHENOTYPE ASSOCIATION OF HbE/β-THALASSEMIA DISEASE AND THE ROLE OF GENETIC MODIFIERS

Sarifah Binti Hanafi¹, Wan Zaidah Abdullah², Rose Adzrianee Adnan³, Rosnah Bahar², Muhammad Farid Johan², Nurul Fatihah Azman², Noor Diana Rashid¹, Siti Aisyah Abdul Ahmad¹, Rosline Hassan², Bin Alwi Zilfalil¹

1. Department of Pediatric, School of Medical Sciences, Universiti Sains Malaysia
2. Department of Hematology, School of Medical Sciences, Universiti Sains Malaysia
3. Human Genome Center, School of Medical Sciences, Universiti Sains Malaysia

Abstract

HbE/β-thalassemia is the most common severe form of thalassemia particularly in SEA region including Malaysia and globally, it comprised of a significant severe form of β-thalassemia disorder. It has various clinical manifestations ranging from very mild anemia to severe manifestation similar to beta thalassemia major. Many different syndromes are observed in HbE/β-thalassemia. Several genetic modifiers have been reported to play important role in contributing to phenotypic variability. The true reasons underlying this phenotypic variability remain unknown. The most reliable predictive factor of the disease phenotype is the nature of the beta globin gene mutation itself. However, the degree of severity is also believed to be affected by other genetic modifiers. For instance, high HbF level ameliorates the clinical severity of β thalassemia patients. Therefore, identification of these genetic modifiers is very important. The association of severe clinical manifestation and the specific β-globin gene mutation has been known. But the wide scope and other potential predictors have been only recently appreciated. This review therefore aimed to reveal the potential genetic modifiers of HbE/β-thalassemia patients based on the previous reported studies. A better understanding on the mechanisms underlying the variety of phenotypes of this disease may lead to the direction for a better future management plans. This also promotes “personalized medicine” in patient care.

Keyword: Thalassemia, Genotype, Phenotype

Corresponding Author: Zilfalil Bin Alwi, Human Genome Center, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kota Bharu, Kelantan, Malaysia
Tel: 09-7676531
Email: zilfalil@usm.my
Genotype-phenotype Association Of HbE/β Thalassemia Disease And The Role Of Genetic Modifiers


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Genetic basis of thalassemia

Thalassemia is characterized by reduced or complete absence of globin chain production and is classified according to the respective affected globin chain. There are mainly 2 types of thalassemias; alpha (α) and beta (β) thalassemias which affects the production of α and β globin chains, respectively. These 2 types of thalassemia also interact each other and influence the clinical presentation. Alpha thalassemia is caused by defective α globin genes (HBA1; haemoglobin subunit alpha 1 and HBA2; haemoglobin subunit alpha 2). HBA1 and HBA2 are arranged in pairs on chromosome 16p. Each of the α globin gene encodes for 1 alpha globin chain in the HbA (α2β2), HbA2 (α2δ2) and HbF (α2γ2) formations [1]. In a normal individual, there are 4 alpha globin genes present (αα/αα).

Majority of α thalassemia is caused by gene deletions and very rarely caused by point mutations. An individual with the loss of one α gene (αα/α-) is almost silent and is called a silent carrier. Loss of 2 genes (-α/α or aa/-) produces mild symptoms, α thalassemia trait. Homozygous for α0 (-/- or α0/α0) have a lethal condition with intrauterine haemolytic anaemia called Hb Bart’s hydropfetalis. This is due to the total lack of α globin chain leading to excessive delta (γ) globin chains which forms Hb Bart (γ4) in the foetus and this Hb Bart is incompatible with life [2]. Meanwhile, an interaction of α+ with α0 causes HbH disease (-/α or α0/α+). In HbH disease, some amount of α-globin chains can still be produced. Thus, it produces an intermediate phenotype and the formation of HbH which consists of 4 beta globin chains (β4) in adult life. Both Hb Bart and HbH have very high oxygen affinity, thus their production is physiologically useless and their precipitations in the red cells cause red cell haemolysis [3].

Beta thalassemia on the other hand, are commonly caused by point mutations and small insertions or deletions of one or two bases on the beta globin gene (HBB; haemoglobin subunit beta) and only a small group of beta thalassemia is caused by gene deletion. Two beta globin chains with 2 alpha globin chains makes HbA (α2β2), which comprises 95% to 97% of haemoglobin in the adult red cell. There are more than 300 mutations of the HBB that had been described, but only a limited few that accounts for the majority of the world wide beta thalassemia mutations. HBB mutations result in quantitative reduction in the output of beta globin chain leading to reduced or complete absence of HbA production which causes thalassemia syndrome. HBB mutations that cause reduced amount of beta globin is called β+ and β++ (milder than β+) thalassemia while mutation that completely inactivates the β globin gene is called βo thalassemia. Usually normal HBB is assigned as ‘β’ without super script. An individual with βo/βo mutation is a beta thalassemia major and typically have severe clinical manifestations. While βo/β+ or β+/β+ are thalassemia intermedia and usually these patients have a diverse clinical severity. Patients with β/βo or β/β+ are thalassemia minor or trait patients and usually are silent carriers [4].

The pathophysiology of beta thalassemia syndrome is mainly caused by excessive α-globin chains in the red blood cells. When only the β globin chain production is affected, there will be excessive amount of alpha globin chains production because they are still normally synthesized. These excessive alpha chains are highly unstable and can precipitate in red cell precursors,
forming intracellular inclusions and also found to associate with the membrane skeletal structure [5]. It has been observed that these beta thalassemic cells have abnormally enhanced cellular oxidant stress due to the production of reactive oxygen species caused by the presence of excessive alpha chains which render the red cells unstable [6,7]. Degradation products of alpha globin chain such as haem and iron also are toxic to the cells. They have shown to directly inhibit a number of cytoplasmic enzymes, further disrupting normal cellular homeostasis which have deleterious effects by binding to the cell membrane proteins and lipids. It is manifested as membrane abnormality and red cell rigidity. Red cell maturation is interrupted and abnormal cells are prematurely destroyed in the bone marrow; a process called ‘ineffective erythropoiesis’. Those red cells which are able to mature enter the peripheral circulation containing these membrane-associated globins which result in reduced deformability of the red cells. Subsequently their passages through the microcirculation in the spleen are interfered resulting in haemolysis [3,5]. Thus, the anaemia in beta thalassemia is due to the combination of ineffective erythropoiesis and haemolysis of red blood cells.

Molecular pathology of HbE/β thalassemia

Due to population migration across continents, the inheritance of hemoglobin disorder has spread worldwide. This phenomenon directly leads to the appearance of diverse type of globin disorders. It is not rare if an individual inherited one mutation from alpha or beta globin allele from one parents, and one mutation is inherited from the other parents that comprised mutated allele from hemoglobin variant. Hemoglobin variants are abnormal forms of hemoglobin produced from the mutation in HBB gene and consequently, leads to the hemoglobin structural defect. Reduced production rate and hemoglobin instability are among the effects of the Hb variant production. These genetic changes will contribute to various pathological effect such as unstable hemoglobin, increase or decrease oxygen affinity, structural change (sickle cell disease) and methaemoglobinemia [3].

Numerous interactions between hemoglobin variants and thalassemia have been reported before. However, only three forms are found to be common which are HbE/β thalassemia, HbS/β thalassemia and HbC/β thalassemia. HbE/β thalassemia is the most frequent compound heterozygous that highly is distributed in Southeast Asia. In 1954, Minnich and colleges reported about this disease where they found the disease in 32 patients of Thailand population [8]. In Malaysia, the disease was firstly reported by Lehman and Singh in 1956 [9].

HbE is a β-chain hemoglobin variant with mild phenotype of β-thalassemia. Individuals who have homozygous form of HbE are clinically normal. However, individuals with compound heterozygous of HbE/β⁺ or HbE/β⁰ thalassemia may have severe clinical manifestation [10]. The pathophysiology of HbE affects the production rate of hemoglobin E. Defects on the βE globin gene dramatically decreased production of βE-globin mRNA and βE-globin chain. This condition arises due to a single base substitution of G to A base at codon 26 which is located in exon 1, resulting in the alteration of Glutamic acid to Lysine. Figure 1 showed correctly and aberrantly spliced βE globin mRNA analysis [11]. The alteration of this amino acid activates a cryptic splice site at codon 25.
thus lowering $\beta^E$ globin chain expression [12]. Therefore, the phenotype for HbE is regarded as $\beta^+$. HbE in the form of heterozygous and homozygous genotype is typically asymptomatic with hypochromic microcytic red cells with mild anemia.

It was believed that, the imbalances of $\alpha/\beta$-globin chain are contributed by several factors. In HbE heterozygous, the decreasing synthesis of $\beta^E$-globin chain leads to the imbalance of $\alpha/\beta$-globin chain synthesis of to 1.2 to 2.1, and affecting on the tertiary conformational HbE molecule. It is also associated with reduction synthesis of $\beta^E$-globin chain. Under oxidative stress condition, HbE is partly unstable and it will be precipitated. Besides that, lower percentage of HbE synthesis is also another factor contributing to the imbalance of accumulation of $\alpha$ and $\beta^E$-globin gene production. Hence, the pathophysiology of the disease is very complicated [13,14].

Oxidative damage, apoptosis, and ineffective erythropoiesis are the main constituents of this disease in which it shortened the life span of the red blood cells. According to Gibbons et al (2001), the association of E hemoglobin variant with $\beta$-thalassemia alleles is the primary basis that contribute to pathophysiologic changes [15]. The mutations caused the imbalance of globin chain to interact resulting in phenotypic variability. However, among the family member with similar mutation, different clinical severity may be displayed [16]. Thus, the true reason for phenotypic variability is still unknown and other genetic modifiers play the role in the clinical variability of HbE/$\beta$ thalassemia.

---

**Figure 1. Simplified representation of aberrant splicing of $\beta^E$-globin mRNA**

$\beta^E$-globin Pre-mRNA

<table>
<thead>
<tr>
<th>Exon 1</th>
<th>Intron 1</th>
<th>Exon 2</th>
<th>Intron 2</th>
<th>Exon 3</th>
</tr>
</thead>
</table>

5' Exon 1 Intron 1 Exon 2 Intron 2 Exon 3 3'

Correctly spliced $\beta^E$–mRNA

Aberrantly spliced $\beta^E$–mRNA

*Black box represents 16 nucleotides at the 3’ end of exon 1 deleted by aberrant splicing
The diversity of HbE/beta thalassemia phenotype and classification

In general, β thalassemia patients can be classified into 3 phenotypes which are; thalassemia major, thalassemia intermedia and thalassemia minor. Patients with β/β0 or β/β+ are classified as thalassemia minor or trait. Apart from being anemic during stressful metabolic events, the natural history of patients with thalassemia minor are typically symptomless. Thalassemia major patients are patients who inherited β0/β0 mutations and are highly transfusion dependent, hence they are also called as transfusion-dependent thalassemia. These children present clinically during the first year of life, as early as 4 to 8 months old, during which the switch from fetal Hb (HbF) to adult Hb (HbA and HbA2) is taking place. The Hb level can be 7g/dl or lower at presentation [17]. There may be evidence of marked erythroid hyperplasia with the typical ‘thalassemic facies’ due to bone marrow expansion of the skull bault and maxillary bones. They usually required blood transfusion in average of 4 weeks or in the range from every 2 to 6 weeks to maintain a steady Hb level of 12 to 12.5g/dl [18].

Thalassemia intermedia patients are compound heterozygotes or homozygous with β0/β+ or β+/β+ mutations. Typically, this group of patients have much severe symptoms than thalassemia minor, but not as severe as thalassemia major. Thus, thalassemia intermedia have a wide spectrum of phenotypes. They usually present at later age than thalassemia major at more than 1 year of age or they even present in late teenager or adulthood. The haemoglobin level at presentation usually ranges from 8g/dl to 10g/dl [18]. On the severe side of the disease spectrum, some may need regular transfusions, developed splenomegaly, signs of marrow expansion and showing growth retardation [19].

The compound heterozygous state of β+ thalassemia and HbE mutation causes both qualitative and quantitative changes to the haemoglobin and HbE/β+ thalassemia patients are classified as thalassemia intermedia. HbE/β+ thalassemia is said to have a ‘modified natural history’ as the patients have a wide range of clinical severity from being asymptomatic with normal growth and development to severely anaemic who are transfusion-dependent [20]. However, most of them have been inaccurately converted to transfusion dependent β thalassemia major. This is because patients first presented with severe anaemia in early childhood. In fact, the anaemia is made worse by a concomitant illness usually secondary to infection. The presenting haemoglobin is usually less than 7g/dl which is similar presentation with beta thalassemia major. They then start receiving transfusion resulting for their life time [21]. But studies have shown that there are HbE/β thalassemia patients who did not require transfusion at all, while some required transfusion only in childhood and still had normal growth and maturity until adulthood [20,22]. Due to this heterogenous and changing phenotype, it has been difficult to develop a straight forward guideline for better management and predict the clinical behavior of these patients control of the disease.

This unique phenotypic diversity of HbE/β thalassemia patients are well-known and have been reported in many populations. Researchers are trying to define the phenotypic variations by classifying them into different severity groups according to their clinical symptoms, signs and
transfusion needs. This approach will provide precise care for patients with different level of disease severity to avoid unnecessary treatment. One of the most comprehensive observational studies was conducted by Premawardhena et al in 2005 to define the phenotypic variability of HbE/β thalassemia patients in Sri Lanka. One hundred and nine (109) patients were followed closely for 5 years and they were classified into 5 groups. Group 1 patients had the most mild symptoms where they present at later age (mean 9.9 years), required no to minimal transfusion, sexually mature in adulthood and had normal growth velocity. Group 2 patients are the same as Group 1 but they required more transfusion than the previous group. Group 3 patients are patients who had splenectomy with better growth and development post splenectomy. Group 4 and 5 patients are on the severe spectrum of the disease where they are transfusion-dependent and had retarded growth and sexual maturity. It is important to note that the mean Hb between the severe and mild groups were only marginally lower with 5.5g/dl and 6.3g/dl (p-value=0.0001) respectively. In short, Premawardhena et al stated that Hb is a very poor indicator of the likely course of HbE/beta thalassemia. They also highlighted that there were lacking of information on the indications for regular transfusion in these patients [20].

Sripichai et al also had developed a scoring system for HbE/βthalassemia patients published in 2008. It was based on 6 independent parameters which are steady Hb state, age at receiving first blood transfusion, requirements for blood transfusion, size of spleen, age at thalassemia presentation and patients’ growth and development. Steady state Hb is the average of Hb levels from previous records before receiving blood transfusion. Requirements for blood transfusion was defined as frequency of transfusion to maintain patient quality of life with rare as being none or once in several years, occasional is every 4 months to once a year and regular is transfusion every 3 weeks to 3 months. Age at thalassemia presentation is the age at which thalassemia symptoms e.g. anaemia, jaundice or splenomegaly appeared in an individual. Growth and development percentile of patients were based on weight and height measurements plotted on a standard Thai growth chart. The first 4 parameters are given scores from a range between 0 to 2 while the last 2 parameters are scored from 0 to 1, giving a potential total score of 10. Patient who scores 0 to 3.5 is considered to have mild disease, 4 to 7 as moderate and 7.5 to 10 as severe. This new scoring system for HbE/β thalassemia disease severity was tested in 950 Thai/Chinese HbE/β thalassemia patients. They found that these 6 parameters were the best combination model where they were all independently associated with disease severity with p-value of less than 0.001 in all parameters [23]. Unfortunately to date, there is no standard classification system that has been applied in clinical practice for management HbE/β thalassemia patients in Malaysia.

**Genetic modifiers of HbE/β-thalassemia**

The diverse of clinical phenotypes of HbE/β thalassemia are believed to be predominantly determined by few factors such as genetic modifiers, access to health-care facilities and environmental factors. Genetic modifiers are the main factors that play a major role in contributing to the remarkable variable phenotype of the disease [24, 25]. Pertaining to this factor, many studies were conducted to reveal the role of genetic modifiers in determining the
severity of the disease. Among the first few studies, HBB was the candidate gene investigated in the past. From the studies, most researchers found their own unique spectrum mutations that correlated to this disease. However, mutations may not be the only molecular alteration that caused different patterns of gene expression. Then, the studies were continued to association with family linkage analysis. Finally, a robust technique known as Genome wide association study (GWAS) was applied to the predict phenotypes from genotypes with certainty. The genetic modifiers of HbE/β thalassemia can be classified as following groups: Primary, β-globin gene mutations in those with underlying β-thalassemia; secondary, loci that involved in globin synthesis and tertiary, loci that are not involved in globin synthesis but might modify the severity of the disease [26].

i) Primary modifier

It has been noted that primary modifier is the most important factor leading to the phenotype variability in β-thalassemia disorder. Primary modifier is the genetic defect or nature of mutation underlying β-globin gene itself [21]. To date, more than 200 of β-globin gene mutations have been reported (http://globin.cse.psu.edu). Most of the mutations are point mutation, small insertion or deletion of 1 or 2 bases along the gene. These diverse mutations are located in several regions of the gene such as promoter, exon, intron, intron-exon boundaries and polyadenylation site [27]. The different locations of the mutations give the different effect on the severity of the phenotype. For instance, mutations that occurred in promoter region influence the mRNA transcription process, resulting in mild phenotype [28].

The mutant alleles can either reduce the synthesis of β-globin chain (β+) or result in complete absence of β-globin chain (β0), thus no HbA production at all [29]. Majority of β0 allele are single base substitution and small insertion or deletion. Defects from the single base substitution in coding sequence of the β polypeptide will produced premature stop codon while, effects of small insertion and deletion lead to the alteration in mRNA reading frame. Similarly, in β+ allele the defect is typically also causes by single base substitution where the new splice site is created [27]. Lists of specific mutations or location for mild β+ and silent mutations were presented in Table 1. In Malaysian Malay population, IVS 1-5 (G-C) and IVS 1-1 (G-T) are the most frequent β-globin gene mutation whereas in Chinese population the most common mutations are CD41/42 (-TCTT), IVS 2-654 (C-D), CD71/72 (+A), CD 17 (A-T) and -28 (A-G). All those mutations appeared to cause completely absence of β-globin chain, except for IVS 1-5 (G-C) and -28 (A-G) which belongs to have β+ phenotype [30, 31]. Figure 2 showed some mutations with specific location in exonic and intronic regions [32].

The interaction of two β+ globin allele which are IVS 1-5 (G-C) and -28 (A-G), caused mild phenotype. Same with a cohort study from Thailand, showing that patients with HbE/β thalassemia have mild clinical symptom. However, co-inheritance of β0 allele with HbE results in widely clinical phenotype, since βE-globin gene mutation leads to alternative splicing of βE-globin mRNA which plays a major role in clinical variability. Hence, severity of the disease can’t be predicted by the β-globin gene mutations alone since, few studies reported that HbE/β0-thalassemia and β0-thalassemia intermedia patients also appear to have mild...
phenotype. All those patients showed the variability in HbF level and this factor may ameliorate the severity of patients with β-thalassemia [22,33].

Table 1. List of HBB gene mutations

<table>
<thead>
<tr>
<th>Mutation type or location</th>
<th>Mild β⁺</th>
<th>Silent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcriptional mutants</td>
<td>-90 (C-T)</td>
<td>-101 (C-T)</td>
</tr>
<tr>
<td>In the proximal CACC box</td>
<td>-88 (C-T)</td>
<td>-92 (C-T)</td>
</tr>
<tr>
<td></td>
<td>-87 (C-A)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-87 (C-G)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-87 (C-T)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-87 (C-A)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-86 (C-T)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-86 (C-G)</td>
<td></td>
</tr>
<tr>
<td>TATA box</td>
<td>-31 (A-G)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-30 (T-A)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-29 (A-G)</td>
<td></td>
</tr>
<tr>
<td>5’ UTR</td>
<td>+22 (G-A)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+10 (–T)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+33 (C-G)</td>
<td></td>
</tr>
<tr>
<td>Alternative splicing</td>
<td>CD 19 (A-C), Malay</td>
<td>CD 27 (G-T), Hb Knossos</td>
</tr>
<tr>
<td>Consensus splicing</td>
<td>IVS 1-6 (T-C)</td>
<td>IVS 2-844 (C-G)</td>
</tr>
<tr>
<td>Intron</td>
<td></td>
<td>+6 (C-G)</td>
</tr>
<tr>
<td>3’ UTR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly-A site</td>
<td>AACAAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AATGAA</td>
<td></td>
</tr>
<tr>
<td>Mild β°-frameshift</td>
<td>CD 6 (-AA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD 8 (–AA)</td>
<td></td>
</tr>
</tbody>
</table>
Non-functional mRNA

a. Nonsense mutants
   CD 43 (G-T)
   CD 35 (C-A)
   CD 15 (A-G)

b. Frame shift mutants
   CD 8/9 (+G)
   CD 15 (-T)

ii. RNA processing mutations
   a. Splice junction changes
      CD 27/28 (+C)
      CD 14/15 (+G)
      CD 71/72 (+T)
      CD 17 (A-T)
      CD 95 (+A)
      CD 41 (-C)
      CD 26 (G-T)
      IVS 1-1 (G-T)
      IVS 1-1 (G-A)

iii. Deletion
   619bp del
   3.5kb del
   12.5kb del
   45kb del
   105bp del
Figure 2. Simplified representation of some HBB mutations
**ii) Secondary modifier**

As previously described, the pathophysiologic changes in patients with HbE/β thalassemia are also determined by the excess number of α-globin chain production. In 1960s, in vitro technique proved that the pathophysiology of the disease is influenced by the globin chain imbalance and the excessive of α-globin chain synthesis [34]. Therefore, it was granted that the secondary modifying factors are controlled by the excess of α-globin chain assembly and degree of globin chain imbalance either through co-inheritance of α-globin gene or via genetic variants that regulated the expression of HbF production [35].

Inheritance of α-globin gene mutation with homozygous or heterozygous β-thalassemia reduced the severity of the disease due to less production of α-globin chain, subsequently improved the degree of α or non-α globin chain imbalance [36]. In contrast, individual inherited triplicated α-globin genes tend to have more severe clinical phenotype due to the increasing degree of α or non-α globin chain imbalance [4]. Meanwhile, the inherited of the two additional α-globin genes in triplicated α-globin genes in homozygous triplicated α-globin genes (aaa/aaa) or heterozygous quadruplicated α-globin genes (aaaa/aa) was found in patients with β-thalassemia minor and was classified as thalassemia intermedia [37,38].

At the stage of α-globin gene formation, the newly-generated α-globin attach to erythroid protein known as Alpha-Hemoglobin-Stabilizing Protein (AHSP) to stabilized itself, then AHSP fold them to form Hb protein. In the study by Kong et al., 2004, animal phenotype was studied and found that the loss of AHSP in knockout mice showed the higher precipitation of α and β-globin leading to the destruction erythropoiesis and worsened the severity of β-thalassemia [39]. According to Lim et al 2012, the expression of AHSP was significantly associated with MCV, HbF, α/β-globin, and excess α-globin production [40].

HbF is one of the indicators which play an important role in disease modifiers as it able to reimburse the deficiency of β-globin chains and HbA. The expression of HbF level is dominated by three different loci: HBG2:g.-158C>T on 11p15.4,BCL11A on 2p16.1 and HBS1L-MYB intergenic region on 6q23.3. It had been reported that, HbF expression in patients with sickle cell anaemia (SCA) was strongly regulated by SNP rs10128556 located at promoter region of HBG1 gene [41], followed by regulation from BCL11A which account for 7-12% in elevating HbF level [42].

Individuals with Hereditary Persistence of Fetal Hemoglobin (HPFH) have the potential to produced high quantity of HbF. This condition is due to the SNP rs11886868 in the BCL11A region that control the production of HbF. Similar effect by this SNP also was seen in patients with homozygotes β⁰-thalassemia, making individual with both diseases appeared to have mild phenotype [43]. In patients with thalassemia intermedia, SNP rs11886868 in BCL11A and SNP rs9389268 in intergenic region of HBS1L-MYB showed the lowest effect in expression of HbF level [42]. However, in SCA and β-thalassemia major, a sequences variation C-T at -158 HbγG promoter region or known as rs7842144, have showed the strongest effect in promoting HbF expression, thus leading to the variability of the phenotype [44-46].
top of that, various SNPs are believed to involve in the regulation of disease severity, however only 3 aforementioned SNPs are reported to show high correlation with remarkable variable phenotype. Further investigation needs to be clarified to search the actual role in modifying the disease severity.

**iii) Tertiary modifier**

Tertiary modifiers are the genetic factors that are not associated with synthesis of globin chain, but may modify the progression of the disease in a different way [47]. For example, factors that influenced the severity of jaundice, iron loading and bone diseased state. Glucuronosyltransferase 1 (UGT1A1) is believed to affect the levels of bilirubin and responsible for gallstones development, hemolysis and ineffective erythropoiesis [48]. While Hemochromatosis gene (HFE) showed correlated with iron overload [49, 50] and for bone disease, the severity is regulated by 3 other different SNP which are Collagen (COL1A1), Vitamin D receptor (VDR) and transforming growth factor beta 1 (TGFB1) [51].

**Conclusion**

The phenotypic heterogeneity of HbE/β thalassemia poses difficulty in classifying and managing HbE/β thalassemia patients. The heterogeneity is largely contributed by genetic variations. Genetic constitution has been widely accepted to play as a key role in affecting thalassemia phenotype. Other than the primary mutation causing the disease, genetic variations such as SNPs that affect the HbF has been widely accepted as a major factor ameliorating thalassemia severity. Hence an understanding of how genetic factors contribute to the disease heterogeneity is very important. With the advances in molecular technologies, studies on association between genetic polymorphisms and thalassemia may have potential clinical application by providing markers of risk, diagnosis, prognosis and possibly therapeutic targets. Personalised medicine is the ultimate aim and we suggest that genetic testing to be included in providing structured patient care programme in the management of HbE/β thalassmia patients in the future.

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Genotype-phenotype Association Of HbE/β Thalassemia Disease And The Role Of Genetic Modifiers


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PLASMA LEAD LEVELS AMONG PAEDIATRIC THALASSAEMIC PATIENTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA, KELANTAN, MALAYSIA

Mohd I Iyen¹, Mohd S Ab Wahab², Norsarwany Mohamad¹, Mariani Mohamad¹, Omotayo O Erejuwa²

1. Department of Paediatrics, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia
2. Department of Pharmacology, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia

Abstract

Objective: Lead toxicity constitutes a major environmental risk to health in both animals and humans of all ages. It is more severe in young children than adults. Blood transfusion is an important source of lead exposure and may predispose premature infants to lead toxicity. Thalassaemia is common in Malaysia and majority of patients require frequent blood transfusion. The objective of this study was to determine whether regular blood transfusion contributed to high blood lead levels in paediatric thalassaemic patients. Method: This was a cross sectional study conducted at the Paediatric Thalassaemia Day Care Unit, General Paediatric Ward and Paediatric Clinic in Hospital Universiti Sains Malaysia (HUSM). A total of 90 patients were included, 45 were thalassaemic transfusion dependant patients and the other 45 were control, who were of the same age and sex with patients group and had never been transfused. The blood samples were taken pre- and post-transfusion for thalassaemic and control groups. Blood lead levels were analyzed using standard Atomic Absorption Spectrometer (AAS) analysis. Results: The overall mean plasma lead levels (2.13 ± 1.72µg/dL) were lower than those of standard CDC recommendations. The independent t-test showed that plasma lead levels in thalassaemic group were significantly (p < 0.05) lower than the levels in controls. However, the ANCOVA analysis revealed the plasma lead levels were not significantly (p > 0.05) different between the two groups. Thus, suggesting that the reduced plasma lead level in thalassaemic group was due to the administration of iron chelators. Increased frequency of blood transfusion also did not significantly (p > 0.05) increase plasma ferritin or lead levels in thalassaemic patients. Conclusion: This study shows that transfusion dependent thalassaemic infants have comparable plasma lead levels to those of age- and sex-matched controls, after taking into consideration the administration of iron chelators.

Keywords: Thalassaemia, Transfusion, Lead, Infants, Paediatrics

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Introduction

Lead toxicity is recognized as a major environmental health risk in both animals and humans of all ages including young children [1, 2]. Lead interferes with several biochemical processes and neurobehavioral functions. Exposure to increased levels of lead is toxic to cells, tissues, organs and systems such as blood, kidneys, heart, reproductive and central nervous systems [3]. In children, this can impair immune function, learning and cause behavioral disorders [4, 5]. The inhalation and ingestion are the common routes of lead exposure. Blood transfusion is an important source of lead exposure and may predispose children and infants to lead toxicity. Blood transfusion in premature infants has been shown to increase blood lead levels which may result in excessively high post-transfusion blood lead levels [6]. Elevated levels of lead were also recently reported in extremely low-birth-weight infants following blood transfusion [7].

Thalassaemia is an inherited blood disorder caused by mutations in globin chain genes, resulting in reduced rate of synthesis of normal α- or β-chains [8]. This lead to unbalanced globin chain synthesis, causing damage to erythroid precursors and thus inducing ineffective erythropoiesis or causing injury to mature erythrocytes and inducing haemolytic anaemia [9]. The majority of thalassaemic patients require frequent blood transfusion. The most common types of thalassaemia include β-thalassaemia, Hb E β-thalassaemia and α-thalassaemia.
come regularly to Paediatric Thalassaemia Day Care Unit for regular blood transfusions. All 54 thalassaemic patients were invited to join this study if they were on regular blood transfusion for at least 8 weekly transfusions or more frequent. The control group was identified by convenience, that is once the samples from the patients group were completed, their age and sex were identified and the control samples were obtained according to the similar age and sex for every single sample from the patients group. The control group was the patients in Paediatric General Ward and Paediatric Clinic who had never been transfused before. Staff nurses and medical students were also invited to be in the control group. Samples were only taken if the carer, patients or volunteers gave their consent for the study. Specimens were then analyzed using AAS (model Spectra AA 220Z). The AAS is a spectro-analytical procedure for the qualitative and quantitative determination of chemical elements employing the absorption of optical radiation (light) by free atoms in the gaseous state. The independent t-test was used to identify significance of difference between two groups. The results of the plasma lead levels were also analyzed using analysis of co-variance (ANCOVA) to exclude the potential role of confounding factor (iron chelators).

**Results**

The demographic results are summarized in Table 1. There were a total of 54 registered transfusion dependent thalassaemic patients in our Paediatric Thalassaemia Day Care Unit, Hospital Universiti Sains Malaysia. Out of this number, 45 patients consented for the study. The other 9 patients were not keen to participate in this study. Control samples were subsequently obtained from volunteers.

**Table 1. Demographic data of respondents**

<table>
<thead>
<tr>
<th>Demographic parameters</th>
<th>Patients group (45 patients)</th>
<th>Control group (45 volunteers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boy</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Girl</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malay:</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td>Chinese:</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>15 (range: 3 to 25)</td>
<td>15 (range: 3 to 25)</td>
</tr>
<tr>
<td>Monthly household income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; RM 1500:</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>&gt; RM 1501:</td>
<td>21</td>
<td>21</td>
</tr>
</tbody>
</table>
Majority of the thalassaemic patients (95.6%) received iron chelating therapy to control their iron overload disease secondary to frequent blood transfusion (Table 2). Iron overload is a known complication for thalassaemic patients. The mean ferritin levels according to their recent iron chelating are also shown in Table 2. None of the participants either from thalassaemic patients group or control group had symptoms of lead poisoning.

### Table 2. Type of chelating agents

<table>
<thead>
<tr>
<th>Type of chelating agents</th>
<th>Number of patients (%)</th>
<th>Ferritin Level (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desferral</td>
<td>32 (71.1)</td>
<td>4811.47</td>
</tr>
<tr>
<td>Deferasirox</td>
<td>7 (15.6)</td>
<td>2774.14</td>
</tr>
<tr>
<td>Deferiprone</td>
<td>4 (8.9)</td>
<td>3020.25</td>
</tr>
<tr>
<td>Not on chelating agents</td>
<td>2 (4.4)</td>
<td></td>
</tr>
</tbody>
</table>

There was no significant ($p > 0.05$) difference in the means between blood lead levels from patients or persons from urban or rural areas. No statistically significant ($p > 0.05$) difference was observed in mean blood lead levels between boys and girls. There was also no significant ($p > 0.05$) difference in the mean blood lead levels...
between patients or persons from low income categories (Table 3).

### Table 3. Blood lead levels based on location, gender and household incomes

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean (SD) (µg/dL)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood lead levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>2.44 (1.65)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Urban</td>
<td>1.78 (1.76)</td>
<td></td>
</tr>
<tr>
<td>Gender:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>2.09 (1.82)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Girls</td>
<td>2.17 (1.64)</td>
<td></td>
</tr>
<tr>
<td>Household incomes:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; RM 1500</td>
<td>2.28 (1.65)</td>
<td></td>
</tr>
<tr>
<td>&gt; RM 1500</td>
<td>1.95 (1.79)</td>
<td>p &gt; 0.05</td>
</tr>
</tbody>
</table>

Majority of the patients (75.6%) were transfused less than 120 times. Increased frequency of blood transfusion did not significantly (p > 0.05) increase plasma ferritin or lead levels in thalassaemic patients (Table 4).

### Table 4. Plasma ferritin and lead levels based on the frequency of blood transfusion among thalassaemic patients

<table>
<thead>
<tr>
<th>Frequency of transfusion</th>
<th>No of patients (%)</th>
<th>Ferritin level Mean (SD)</th>
<th>Lead level Mean (SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 – 60</td>
<td>7 (15.6%)</td>
<td>2637.3 (619.3)</td>
<td>1.41 (0.71)</td>
<td></td>
</tr>
<tr>
<td>61 – 90</td>
<td>14 (31.1%)</td>
<td>3856.4 (2489.3)</td>
<td>1.04 (0.75)</td>
<td></td>
</tr>
<tr>
<td>91 – 120</td>
<td>13 (28.9%)</td>
<td>3996.5 (1499.6)</td>
<td>1.20 (1.15)</td>
<td></td>
</tr>
<tr>
<td>121 – 150</td>
<td>5 (11.1%)</td>
<td>5368.6 (2806.3)</td>
<td>1.14 (0.63)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>151 – 180</td>
<td>0 (0.0%)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>181 – 210</td>
<td>4 (8.9%)</td>
<td>6380.3 (3277.2)</td>
<td>1.28 (0.53)</td>
<td></td>
</tr>
<tr>
<td>211 – 240</td>
<td>2 (4.4%)</td>
<td>5945.0 (3389.9)</td>
<td>0.35 (1.06)</td>
<td></td>
</tr>
</tbody>
</table>

In thalassaemic patients group, the mean blood lead level (1.14 ± 0.85 µg/dL) was significantly (p < 0.05) lower than that of the control group (3.12 ± 1.81 µg/dL) (Figure 1). However, both mean values were within the safe blood lead level range as outlined in the current standard of CDC recommendations (i.e < 10µg/dL).
Discussion

Lead poisoning in children is a global health problem that may impair learning and diminish intelligence [13]. A total of 90 blood samples, obtained from the major ethnic groups in Kelantan, Malaysia were evaluated for lead levels. The demographic data were equally distributed, based on sex and age, between patient and control groups. Majority of the thalassaemic patients in this study had Hb E β-thalassaemia (84.4%) and the rest were Hb β -thalassaemia major patients. This is consistent with previous findings which showed that Hb E β -thalassaemia is the commonest type of thalassaemia in Asia especially in South East Asian countries such as Thailand, Cambodia and Laos [14-16]. Thalassaemia major is characterized by progressive iron overload secondary to ineffective erythropoiesis and regular blood transfusion, coupled with the lack of physiological mechanism for excreting excess iron. In regularly transfused patients, iron accumulation occurs at the rate of approximately 0.5mg/kg/day [17]. Iron overload is usually treated or managed by chelating therapy [14].

In this study, desferral, deferiprone and deferasirox were among chelating agents used. Desferral was the most commonly prescribed (71.1%), followed by deferasirox (15.6%) and deferiprone (8.9%). Chelators were not prescribed to two patients as result of young age (3 years old) and low ferritin level (<1000 µmol/L). Varied effects of chelating agents on ferritin levels were observed. This may be due to lack of compliance. In spite of its limitations, serum ferritin level still remains an important marker to monitor the iron overload in thalassaemic patients [18]. The high ferritin level in thalassaemic patients is an important finding due to known interaction between iron and lead which may affect the lead levels in these thalassaemic patients [19].
The independent t-test showed that the thalassaemic patients group had significantly \( (p < 0.05) \) lower plasma lead levels than the controls. However, both mean values are much lower than the current standard CDC recommendations for lead poisoning (> 10µg/dL).

The lower plasma lead level in thalassaemic patients may be due to a number of factors. It may be a consequence of elevated ferritin levels in the thalassaemic patients. Low level of iron has been shown to increase lead toxicity [20-21]. Uptake of lead by erythrocytes and intestinal absorption of lead are also decreased in the presence of iron. This effect seems to be mediated by a common binding protein in the intestinal mucosa with greater affinity for iron than for lead [22]. Recently, studies have also corroborated this beneficial effect of iron on lead toxicity. Wang and colleagues showed that iron salt supplementation in rats reduce lead levels in blood and organs including liver and kidney [23]. A new study found that exposure to lead significantly reduced haemoglobin level among workers of oil refinery [24]. These findings generally indicated that deficiency or low level of iron may enhance elevated plasma lead level; and thus suggesting that iron overload may in turn cause low lead absorption or low blood lead level.

The lower plasma lead level in thalassaemic patients may also be due to differences in lead half-life in different body compartments. For instance, lead has an half-life of about 25-40 days in erythrocytes, 40 days in soft tissues whereas it can be as long as 28 years in bones [25]. Thalassaemia is characterized by defective erythropoiesis and/or increased haemolysis. The process of haemolysis might increase excretion of blood lead levels but may not reflect the actual lead level in body. Hence, future studies involving thalassaemic patients may require other modes of lead screening.

Considering that iron chelators are known to chelate lead as well, the administration of iron chelators might contribute to the lower plasma lead levels in thalassaemic patients. In order to rule out the contributory role of iron chelators, the data were further analysed using ANCOVA. The ANCOVA analysis did reveal that the plasma lead levels were not significantly \( (p > 0.05) \) different between the thalassaemic patient and control groups. This lack of significant difference suggests the reduced plasma lead levels observed in thalassaemic patients is partly a consequence of administered iron chelators.

Other yet-to-be identified nutritional and environmental factors may also contribute to the lower levels of blood lead in thalassaemic patients. In future studies, it may be important to determine the amount of lead administered in each transfusion by measuring the blood lead levels in blood donors and in pre-transfusion thalassaemic patients. Based on known half-life of lead in erythrocytes (i.e 25-40 days), blood lead levels may be re-evaluated around 3 weeks post-transfusion or before next blood transfusion in these thalassaemic patients. Factors aggravating or inhibiting the lead levels in this group of patients are still unclear and are open for further studies.

In conclusion, this pilot study has provided evidence indicating that, like other children in our population, our thalassaemic patients who are transfusion dependent are not exposed to excessively elevated levels of blood lead. The data further revealed that the low plasma lead levels in thalassaemic patients were due to the administration of
iron chelators which probably chelated lead as well. More detailed studies that take into consideration some of the aforementioned suggestions or recommendations including investigating the exact interaction between iron overload or iron chelators and blood lead level in thalassaemic patients are required.

**Acknowledgements**

This study was fully sponsored by the Universiti Sains Malaysia short term grant (USMKK/PPP/JEPEM 199.4(1.8)). The authors would like to thank the children, parents, Universiti Sains Malaysia and all individuals who were directly or indirectly involved in this study.

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Plasma Lead Levels Among Paediatric Thalassaemic Patients In Hospital Universiti Sains Malaysia, Kelantan, Malaysia

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ORIGINAL ARTICLE

RISK FACTORS FOR CHILDHOOD PNEUMONIA IN NORTH EASTERN PAKISTAN: A CASE-CONTROL STUDY

Sommayya Aftab¹, Iftikhar Ejaz¹, Umar Waqar¹, Humayun Iqbal Khan¹, Asif Hanif¹, Amir Usman³, Asma Mushtaq¹, Ilyas Muhammad Nadeem², Holifa Saheera Asmara², Atif Amin Baig³

¹. Pediatric Medical Unit III, The Children's Hospital & Institute of Child Health (ICH) Lahore, 54000, Pakistan
². Faculty of Medicine, Universiti Sultan Zainal Abidin, 20400 Kuala Terengganu, Terengganu, Malaysia
³. University of Lahore, 54000, Pakistan

Abstract

Background: Pneumonia is defined as the inflammation of parenchyma of the lung. It is a substantial cause of morbidity and mortality in childhood throughout the world. The incidence of pneumonia in children under the age of five years is 0.29 episodes per child-year, which equates 151.8 million cases annually in developing countries. Objective: To determine the risk factors for complicated pneumonia. Material and Methods: This case-control study conducted in Medical Unit III, The Children’s Hospital & Institute of Child Health Lahore. Out of total of 180 cases of pneumonia, 100 were labeled as complicated pneumonia (case) and 80 were labeled as uncomplicated pneumonia (control). Complicated pneumonia included pneumonia with associated complications. Detail history was taken in both groups and recorded on predesigned proforma. Data was analyzed by SPSS 20. Quantitative risk factors like child age, maternal age and father age were analyzed by mean and standard deviation. However qualitative risk factors like method of feeding, malnutrition, immunization, anaemia, and non-vaccination were analyzed by applying chi-square test and finding odd ratios. Results: Most significant risk factors associated with complicated pneumonia included younger age, maternal and father education, rural area, malnutrition, anaemia, rickets, birth problems, admission during neonatal life due to pneumonia, bottle feeding, non-vaccination, referral and delayed in presentation (p <0.05). Conclusion: Important risk factors for complicated pneumonia like malnutrition, anemia, rickets, birth disaster, admission during neonatal life due to pneumonia, bottle feeding, non-vaccination, referred cases and delayed in presentation can be reduced by improving child health education and immunization.

Keywords: Complicated Pneumonia, Uncomplicated Pneumonia, Risk Factors, Developing Countries

Corresponding Author: Associate Professor. Dr. Atif Amin Baig, Faculty of Medicine, Universiti Sultan Zainal Abidin, Terengganu, Malaysia
Tel: +6096275646
Email: atifamin@unisza.edu.my

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Introduction

Pneumonia is defined as the inflammation of parenchyma of the lung. It is a substantial cause of morbidity and mortality in childhood throughout the world [1]. The incidence of pneumonia in children under the age of five years is 0.29 episodes per child-year, which equates 151.8 million cases annually in developing countries [2]. Childhood pneumonia remains as a leading killer of children in developing countries, where it accounts for up to 21% of deaths in children under the age of five years [3]. An estimated 1.9 million children die from pneumonia yearly [4]. Cough is the most common symptom of pneumonia, along with tachypnea, retractions and hypoxemia. These may be accompanied by congestion, fever, irritability and decreased feeding [5]. Common complications include pleural effusion, empyema, pneumothorax, pericarditis and sepsis. Meningitis, suppurative arthritis and osteomyelitis due to bacteremia are rare complications [1].

Of all the pneumonia cases occurring in countries with high incidence, 8.7% are severe enough to be life threatening [2]. Multiple studies have been done to identify the risk factors for complicated or severe pneumonia. These risk factors included demographic data, birth milestone, nutrition status, past co morbidities, vaccination status, maternal vaccination, environmental and social factors, water source, delay in presentation etc. [6,7].

Studies on the risks factors for complicated pneumonia in Pakistan are few in the literature. The purpose of conducting this study is to determine the risk factors of complicated pneumonia in developing countries like Pakistan. The controlled and modified control of the risk factors can be used to reduce the mortality and morbidity in children suffering from pneumonia in Pakistan.

Methodology

This prospective case-control study was conducted at the Medical Unit III, The Children Hospital & Institute of Child Health Lahore from October 2013 to March 2014 after approval from ethical committee of the institute. One hundred and eighty cases of chest X-ray proven pneumonia age >1 month to 5 years, both male and female genders were included in this study. Cases of pneumonia with any chronic ailment (chronic liver disease, chronic kidney disease, congenital and acquired heart disease, cerebral palsy (CP), neurodegenerative disorder, malignancies and known aplastic anaemia) were excluded from the study. Complicated pneumonia included pneumonia with any one of the following i.e. empyema, pleural effusion pneumothorax, sepsis, severe sepsis and septic shock. Empyema and pleural effusion was confirmed by ultrasonography of chest and diagnostic tap while pneumothorax was confirmed on chest X-ray. Sepsis included pneumonia with any two systemic inflammatory response syndrome (SIRS), severe sepsis included sepsis plus one of the following: cardiovascular instability or acute respiratory distress syndrome or two or more of other organ dysfunctions and septic shock included hypotension persisting despite adequate fluid resuscitation. Uncomplicated pneumonia (viral and consolidation) included pneumonia with no above mentioned complication. After informed consent by parents detail history and examination regarding multiple risk factors of complicated pneumonia were evaluated in two groups and recorded on predesigned pro forma. These risk factors included demographic data: age, gender, living area; risk factors related to child: malnutrition, presentation direct or referred, treatment
before presentation, type of treatment before presentation, delay in presentation, place of delivery, gestation age, adverse birth events, neonatal admission due to respiratory tract infection, breast feeding trend, vaccination status, anaemia, rickets; risk factors related to family: maternal age, maternal education, father age and father education. Data were analyzed by SPSS 20. Quantitative data like child age, maternal age and paternal age were compared by calculating means and standard deviation. However, the rest of all risk factors were compared in two groups by applying chi-square test and calculating Odd ratios (OR). P value <0.05 was taken significant with 95% confidence interval.

Results

Out of 180 cases presented at Children Hospital and Institute of Child Health Lahore, 100 cases were found to be complicated pneumonia (case) and 80 were found to be uncomplicated (control). In this study the mean age of children was 19.98 ± 16.49 months with minimum age 1 month and maximum age was 67 months. There were 123 (68.3%) male and 57(31.7%) female patients with male to female ratio 2.16:1. The frequency of uncomplicated pneumonia was 44.4%. The mean age of patients with complicated and uncomplicated pneumonia was 11.80 ± 11.59 months and 30.21 ± 16.03 months respectively, p-value ≤ 0.001. There were 73% male with complicated pneumonia and 62.5% with uncomplicated pneumonia. The frequency of female patients with complicated and uncomplicated pneumonia was 27% and 37.5% respectively, no association was observed in type of pneumonia and gender, (p-value = 0.132). The mean mother’s and father’s age were 26.38 ± 4.15 years and 30.73 ± 4.15 respectively as shown in Table 1. There was no association between type of pneumonia and mother’s and father’s age ≥ 35 years (p-value > 0.05). Moreover type of pneumonia was associated with mother’s and father’s education ≤ 5 years of education, (p-value < 0.001). An OR analysis revealed that there were 26.31 and 44.53 times higher chances of complicated pneumonia when mother’s and father’s education was ≤ 5 years.

Table 1. Descriptive statistics of age of patients and their parents

<table>
<thead>
<tr>
<th></th>
<th>Age (Months)</th>
<th>Mother’s Age (years)</th>
<th>Father’s Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>19.98</td>
<td>26.38</td>
<td>30.73</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>16.495</td>
<td>4.150</td>
<td>4.156</td>
</tr>
<tr>
<td>Minimum</td>
<td>1</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>Maximum</td>
<td>60</td>
<td>37</td>
<td>41</td>
</tr>
</tbody>
</table>

In patients with complicated pneumonia there were 64% patients belonged to rural and 36% belonged to urban and among patients with uncomplicated pneumonia there were 42.5% rural and 57.5% were urban. There was significant association between type of pneumonia and living area with 2.4 times more chances of complicated pneumonia with rural living status as described in Table 2.
### Table 2. Bivariate analysis types of pneumonia and different risk factors

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Complicated (n=100)</th>
<th>Uncomplicated (n=80)</th>
<th>Chi-square (p-value)</th>
<th>Odds Ratio</th>
<th>95% C.I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>73 (73%)</td>
<td>50 (62.5%)</td>
<td>2.64 (0.132)</td>
<td>1.62</td>
<td>0.682 – 3.05</td>
</tr>
<tr>
<td>Female</td>
<td>27 (27%)</td>
<td>30 (37.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother’s age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;35</td>
<td>10 (10%)</td>
<td>3 (3.75%)</td>
<td>2.59 (0.107)</td>
<td>2.85</td>
<td>0.75 – 10.73</td>
</tr>
<tr>
<td>≤35</td>
<td>90 (90%)</td>
<td>77 (96.25%)</td>
<td></td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Father’s age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤35</td>
<td>80 (80%)</td>
<td>70 (87.5%)</td>
<td>(0.180)</td>
<td>1.75</td>
<td>0.768 – 3.99</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>12 (12%)</td>
<td>37 (46.25%)</td>
<td>(≤0.001)</td>
<td>2.85</td>
<td>1.32 – 5.53</td>
</tr>
<tr>
<td>Living area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>64 (64%)</td>
<td>34 (42.5%)</td>
<td>8.28 (0.004)</td>
<td>2.4</td>
<td>1.32 – 4.39</td>
</tr>
<tr>
<td>Urban</td>
<td>36 (36%)</td>
<td>46 (57.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery other than hospital</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>89 (89%)</td>
<td>63 (78.75%)</td>
<td>3.55 (0.059)</td>
<td>2.18</td>
<td>0.986 – 4.98</td>
</tr>
<tr>
<td>No</td>
<td>11 (11%)</td>
<td>17 (21.25%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm</td>
<td>8 (8%)</td>
<td>4 (5%)</td>
<td>0.63 (0.423)</td>
<td>1.65</td>
<td>0.48 – 5.70</td>
</tr>
<tr>
<td>Term</td>
<td>92 (92%)</td>
<td>76 (95%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malnutrition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>76 (76%)</td>
<td>18 (22.5%)</td>
<td>50.98 (≤0.001)</td>
<td>6.31</td>
<td>2.99 – 13.31</td>
</tr>
<tr>
<td>No</td>
<td>24 (24%)</td>
<td>62 (77.5%)</td>
<td></td>
<td>10.9</td>
<td>4.65 – 18.70</td>
</tr>
<tr>
<td>Anemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>81 (81%)</td>
<td>16 (20%)</td>
<td>66.65 (≤0.001)</td>
<td>2.56</td>
<td>1.18 – 5.53</td>
</tr>
<tr>
<td>No</td>
<td>19 (19%)</td>
<td>64 (80%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adverse birth event</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>29 (29%)</td>
<td>11 (13.75%)</td>
<td>5.98 (≤0.014)</td>
<td>2.56</td>
<td>1.18 – 5.53</td>
</tr>
<tr>
<td>No</td>
<td>71 (71%)</td>
<td>69 (86.25%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission during neonatal age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>41 (41%)</td>
<td>3 (3.75%)</td>
<td>33.39 (≤0.001)</td>
<td>17.8</td>
<td>5.26 – 60.43</td>
</tr>
<tr>
<td>No</td>
<td>59 (59%)</td>
<td>77 (96.25%)</td>
<td></td>
<td>3</td>
<td>60.43</td>
</tr>
<tr>
<td>Feeding method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottle</td>
<td>32 (32%)</td>
<td>14 (17.5%)</td>
<td>4.91 (0.027)</td>
<td>2.21</td>
<td>1.08 – 4.5</td>
</tr>
<tr>
<td>Breast</td>
<td>68 (68%)</td>
<td>66 (82.5%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Non-vaccination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>48 (48%)</td>
<td>52 (65%)</td>
<td>34.71 (≤0.001)</td>
<td>11.3</td>
<td>4.54 – 28.56</td>
</tr>
<tr>
<td>No</td>
<td>66 (66%)</td>
<td>74 (92.5%)</td>
<td></td>
<td>8</td>
<td>28.56</td>
</tr>
<tr>
<td>Rickets diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13 (13%)</td>
<td>2 (2.5%)</td>
<td>6.41 (≤0.011)</td>
<td>5.83</td>
<td>2.63 – 13.63</td>
</tr>
<tr>
<td>No</td>
<td>87 (87%)</td>
<td>78 (97.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Referral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Referred</td>
<td>44 (44%)</td>
<td>17 (21.25%)</td>
<td>10.27 (≤0.001)</td>
<td>2.91</td>
<td>1.49 – 5.66</td>
</tr>
<tr>
<td>Direct</td>
<td>56 (56%)</td>
<td>63 (78.75%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>93 (93%)</td>
<td>34 (42.75%)</td>
<td>54.56 (≤0.001)</td>
<td>17.4</td>
<td>7.04 – 43.63</td>
</tr>
<tr>
<td>No</td>
<td>7 (7%)</td>
<td>46 (57.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode of admission</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPD</td>
<td>0 (0%)</td>
<td>17 (21.25%)</td>
<td>23.46 (≤0.001)</td>
<td>2.58</td>
<td>2.13 – 3.14</td>
</tr>
<tr>
<td>Emergency</td>
<td>100 (100%)</td>
<td>63 (78.75%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>16 (16%)</td>
<td>60 (75%)</td>
<td>(≤0.001)</td>
<td>5</td>
<td>32.88</td>
</tr>
</tbody>
</table>

*OPD: Out-Patient Department*
The frequency of delivery other than hospital was seen 89% in complicated pneumonia and 78.75% in uncomplicated pneumonia with insignificant association and (OR of 2.18, p-value > 0.05). There were significantly higher chances (OR =10.90) of complicated pneumonia for malnutrition of children and there were 17.053 times more chances for complicated pneumonia (OR = 17.053) with significant association as well, p-value < 0.001 as shown in Table 2.

Furthermore, we also found significant association of complicated pneumonia with adverse birth event (OR=2.56), admission during neonatal age (OR=17.83), bottle feeding (OR=2.21), non-vaccination status (OR= 11.38), rickets (OR=5.83), referral from other hospitals (OR=2.91), prior treatment of pneumonia (OR=17.40), admission through emergency (OR= 2.58) and late admission of the patients (OR=15.75) as described in Table 2.

Discussion

Pneumonia is the leading cause of mortality in children < 5 years of age worldwide. The incidence in this group is 156 millions episodes per year, of which 151 millions are in developing countries [2]. With the inclusion of neonatal pneumonia, recent estimates indicate that pneumonia is the largest contributor to child mortality, accounting for 28-34% of all under 5 deaths globally. The bulk of death due to pneumonia is from developing countries because they have higher exposure rates to risk factors for developing Acute Respiratory Infection (ARI) [8].

In present study we evaluated the risk factors (Figure 1) that can lead to complicated pneumonia. In this study younger age was found to be a significant risk factor associated with complicated pneumonia. At younger age the children have small airways and immature defense system that predispose them to these complications [9,10,11,12]. Some authors in contrast found no relationship between age and complicated pneumonia [13,14,15,16].
In our study no association was observed between gender and type of pneumonia. In literature there is no consensus about relationship between gender and complication in pneumonia, some authors suggest no association [13,17,18], while others suggest it is common in male [11,12,19] and others found it common in the female [15].

In present study malnutrition, incomplete vaccination, delay in presentation and residency in rural area are important risk factors for complicated pneumonia in childhood. This association was also observed by Rudan et al. 2008 [2]. Severe malnutrition can lead to fatal pneumonia and death was also observed by Naheed et al. 2009 [20].

In this study bottle feeding is associated with complicated pneumonia. Many authors also support that lack of breastfeeding is associated with complicated pneumonia [11,18].

In our study it was observed that place of delivery, mode of delivery and gestational
age is not associated with complication in pneumonia. In contrast to our finding some authors found prematurity being an important risk factor for complicated pneumonia later in life [9,11,19,19]. We also observed that admission in neonatal age due to pneumonia was associated with complicated pneumonia later in life. This finding was also supported by Mello et al. 2006 [21].

We found that anaemia and rickets were more common in complicated pneumonia than uncomplicated one. This association of complicated pneumonia and anaemia was also observed by Wexler, et al. 2006 [22]. Relationship between rickets and severe or complicated pneumonia in children was also suggested by Roth, et al. (2008) [23]. We observed that poor maternal and paternal education strongly associated with complicated pneumonia. In present study most of cases of complicated pneumonia came from rural area which seems to be an important reason for their delay in presentation. It was observed that many cases of complicated pneumonia were referred and taken treatment before presentation. This finding was also supported by Khan et al. 2009 [24].

**Conclusion**

We observed that malnutrition, incomplete vaccination, delay in presentation, anaemia, rickets, poor parental education, adverse birth event and bottle feeding are important risk factors for complicated pneumonia. By modifying these factors we can reduce the mortality and morbidity in these children, which can be possible by improving our vaccination (EPI), promoting child health care education and improving primary health care.

**References**


ORIGINAL ARTICLE

RETROSPECTIVE STUDY ON NEONATES OF DENGUE POSITIVE MOTHER OVER A PERIOD OF ONE YEAR

Chang Jia Vern, Lim Kang Yaik, Ang Ee Lee

Paediatric Department, Hospital Tengku Ampuan Rahimah Klang, Selangor, Malaysia

Abstract

Objectives: This is a retrospective study in which all neonates with confirmed dengue fever mother admitted to our special care nursery from March 2014 to March 2015 were recruited. This is to determine the percentage of positive dengue serology in the neonates of mother with confirmed dengue fever and to investigate the correlation between the duration of maternal illness with these neonatal seropositivity of dengue antibody and their presenting symptoms. Method: A total of 22 neonates whose mother with confirmed dengue fever were recruited out of which 14 (63.6%) neonates of confirmed dengue fever mothers had positive dengue serology. Results: Eight out of fourteen neonates were dengue seropositive when mother dengue illness was between day 1 to 5 of illness (acute phase) at the time of delivery. Thirteen out of twenty-two neonates (59%) were symptomatic, out of which 61.5% (8 out of 13) were delivered during maternal acute phase of illness. Conclusion: From this study, we concluded that neonates were more likely to be dengue seropositive and symptomatic when mother presented in acute phase of illness during delivery. However, our sample size was small, only 22 neonates were recruited from a single centre, therefore a larger sample size from multicentre is required in future.

Keywords: Neonatal Dengue, Dengue, Neonatal

Corresponding Author: Dr. Chang Jia Vern, Paediatric Department, Hospital Tengku Ampuan Rahimah, Taman Chi Lung, Jalan Langat, 41200 Klang, Selangor, Malaysia
Tel: +60172672886
Email: changjv0102@gmail.com

Introduction

Dengue is a result of dengue virus infection, an RNA virus (flavivirus), transmitted by Aedes aegypti mosquitoes [1]. Infection by any one of the 4 recognized serotype does not confer immunity to the other three [1]. Prior infection by one serotype (primary dengue) predisposes a person to more severe infection manifestations by other serotypes (secondary dengue) [2]. With infections rising to epidemic proportions, even pregnant women alike are not spared thus giving rise to increasing perinatal transmission of dengue fever [3]. Maternal dengue results in increased maternal mortality, low birth weights, preterm delivery, neonatal admissions and fetal death. Neonatal infection give rise to a spectrum of outcomes ranging from...
asymptomatic to death [4,5].

This study aimed to determine the percentage of positive dengue serology (Dengue NS-1 or IgM) amongst neonates of mother with confirmed dengue fever (positive Dengue NS-1 or IgM), diagnosed in Hospital Tengku Ampuan Rahimah (HTAR), Klang from March 2014 to March 2015; and to investigate the correlation between the duration of maternal illness with symptoms of neonatal infection and seropositive test results.

**Methodology**

An observational study was carried out whereby, 22 neonates of mothers with confirmed dengue (positive Dengue NS-1 or IgM), admitted to our special care nursery in HTAR, Klang, from March 2014 to March 2015, were recruited and reviewed in retrospect. Data was collected and analyzed.

**Results**

A total of 22 neonates of mothers with confirmed dengue fever were recruited. Fourteen out of twenty-two neonates (63.6%) were tested positive, as shown in Table 1.

**Table 1. Positive dengue serology in neonates of mothers with confirmed dengue fever**

<table>
<thead>
<tr>
<th>Dengue serology</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>8</td>
<td>36.4</td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>63.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>22</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

**Figure 1. Percentage of positive dengue serology in neonates of mother with confirmed dengue fever**

- **63.6%** Positive
- **36.4%** Negative

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Table 2. Common presenting symptoms in neonates of mothers with dengue fever

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>4</td>
<td>18.2%</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>6</td>
<td>27.3%</td>
</tr>
<tr>
<td>Fever and thrombocytopenia</td>
<td>3</td>
<td>13.6%</td>
</tr>
<tr>
<td>Renal and liver involvement</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>No Symptoms</td>
<td>9</td>
<td>40.9%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>22</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

Thirteen out of twenty-two neonates (59.1%) were symptomatic. Most common presenting symptoms were fever, thrombocytopenia or fever with thrombocytopenia, which accounts for 18.2%, 27.3% and 13.6% respectively (Figure 2 and Table 2).
Out of 14 neonates who tested positive, 8 (36.3%) were delivered to mothers at acute phase (day 1 to day 5 of illness) (Figure 3).

From Figure 4, out of the total 22 neonates, 36.3% of the neonates born by mother during acute phase of illness were symptomatic; as compared to those who were born by mother at recovery phase of dengue and those who had previous infection, only 4.5% were symptomatic respectively.

Only one neonate within the acute phase group remained asymptomatic (4.5%).
A majority of those who were symptomatic, 8 out of 13 (61.5%), were from the group presenting during acute phase of maternal illness (Figure 5).

Discussion

In this study, the type of dengue serology tests that were done for mothers and neonates were by NS-1 or IgM ELISA method.

The higher percentages of neonates who were seropositive, were in keeping with the increasing numbers of vertical transmission, as a result from the emergence of the dengue epidemic.

Of all known presentation of dengue, there is no significant renal or liver impairment noted in our study group (Table 2). This may be due in part to the small sample size involved which may not reflect the true
presentation within the general population or due to the difference in the virulence of the strain involved.

As shown in Figure 3, those delivered to mothers with prior infections, were more likely to be seropositive from maternal transplacental dengue antibodies (IgG) [6,7]. A majority presented within acute phase of maternal infection reflecting the greater risk of vertical transmission during this period of maternal illness where viral load is at its peak (viraemic phase) [6,7].

**Figure 7. Course of Dengue Illness**

Two cases from the recovery group and later phase of illness were symptomatic. This could be due to neonatal pyrexia following maternal postpartum fever, which reflects postnatal sepsis rather than dengue itself (Figure 4).

Those neonates born within the acute phase of maternal illness were more likely to present with symptoms probably due to the lack of attenuation from transplacental maternal antibodies. Neonates of mothers in later stages of illness or having prior infection may have had intrauterine infection and already entered recovery phase upon delivery and are thus asymptomatic [8].

The isolated case from the maternal acute phase group which remained asymptomatic was probably due to a milder maternal dengue infection or probably a primary dengue infection at presentation.

From previous observations, the time of acquiring dengue virus intrauterinely could affect the fetus, whereby 2\textsuperscript{nd} trimester infection could lead to premature labour and fetal death. Infection near, or at, term posed little effect on the infants [4,5]. The above adverse pregnancy outcomes reported was not seen in our present study. All neonates of different gestational ages had good outcome. However, one of the premature neonate had poor outcome and eventually died on day 8 of life. This may be due to extreme low birth weight and extreme prematurity which led to severe sepsis rather
than dengue itself (Figure 6).

These observations may not have reflected the true population presentation due to the small sample size of this study. A larger studies with larger sample sizes are necessary to demonstrate a more accurate observation and this can be achieved with multi-centered studies.

**Conclusion**

From this retrospective study, we can conclude that neonates of mothers who were dengue positive will be seropositive, due transplacental dengue antibodies transfer (IgG). They were also more likely to be dengue seropositive and symptomatic when born to mothers in acute phase of illness due to lack of immunoglobulin protection and peak levels of maternal viraemia. Therefore, neonates of mother with confirmed dengue fever should be admitted for closer monitoring, especially those delivered during maternal acute phase of dengue illness.

Studies with larger sample size from multiple centres are required in future to better illustrate the presentations and validate such findings with better accuracy.

**References**


CASE REPORT

TUBERCULOSIS OF THE RIB IN A 20 MONTH’S OLD BOY

El Mouhtadi Aghoutane, Tarik Salama, Redouane El Fezzazi

Pediatric surgery department, Kadi Ayyad University, Marrakech, Morocco

Abstract

Primary tuberculosis osteomyelitis of the rib is rare. The majority of cases occur in children and young adults and there is difficulty in diagnosis mainly in young children. We report a new rare case in a child aged only of 20 months causing rib destruction. Tuberculosis was confirmed on histological examination. No lesions in lung parenchyma or lymphadenopathy were associated. The patient was successfully managed by anti-tubercular drugs.

Keywords: Tubercular Osteomyelitis, Rib Destruction, Children

Corresponding author: El Mouhtadi Aghoutane, Pediatric surgery department, CHU Mohammed VI, Kadi Ayyad University, Marrakech, Morocco
Tel: 00212611792289
Fax: 00212524306869
Email: elmohtadi@yahoo.com

Introduction

Despite the decline in the incidence of tuberculosis during the last decades, the disease remains a significant public health problem in developing countries like Morocco [1].

Musculoskeletal tuberculosis accounted for 15% of all extra pulmonary localizations [1-2]. Tuberculosis of the rib is an uncommon form of osteoarticular tuberculosis, and it occurs in 0 – 5% of cases of bone and joint infection [3]. It has an insidious onset and < 50% of patients have active pulmonary disease [4]. We report a new case of rib tuberculosis in a child aged only of 20 months, with literature review.

Case Report

A 20 months old boy of low socioeconomic status presented with 3 months history of pain and swelling over right chest wall. There was no family history of previous exposure to tuberculosis infection. The boy received BCG vaccination at birth. Physical examination showed normal weight and temperature. On his chest examination, swelling in the right chest was 2/2 cm. It was tender to palpation with normal local temperature. Abdominal and pulmonary examinations were normal. Laboratory investigations showed Hb -12g/dL, erythrocyte sedimentation rate 40mm/1h. A tuberculin skin test was positive (15/12mm) after 48 hours of test dose. Chest radiograph showed a lytic lesion of the anterior part of the right seventh rib (Figure 1).
Figure 1. Chest radiograph showed a lytic lesion of the anterior part of the right seventh rib.

The rest of the lung parenchyma was normal. Contrast enhanced computed tomography of the chest showed an expansive lytic lesion involving the anterior part of the seventh rib with cortical erosion and destruction of the same rib. However, there was no evidence of mediastinal or hilar lymph node involvement. Lung window on computed tomography was normal (Figure 2).
Surgical debridement of necrotic tissues and drainage of purulent liquid were performed. Tuberculosis of the rib was confirmed on histological examination of the resection specimens by the presence of a caseous necrosis with epithelioid and langerhans giant cells. Anti-tuberculosis drugs were administered for 12 months. The boy was followed-up for 17 months with satisfactory healing of his tuberculosis lesions.

Discussion

Musculoskeletal tuberculosis is the commonest form of extra pulmonary tuberculosis which accounts for 10 – 15% of all the tuberculosis cases in developing world. While in western world it accounts for only 1 – 2% of cases [5]. In skeletal system tuberculosis, vertebral column (50%) is the commonest site followed by hip (15%) and knees (5%). Involvement of the rib in skeletal tuberculosis is being reported from 0 to 5% of the bone tuberculosis and it is the commonest inflammatory disorder involving the rib [6]. Majority of the cases occur in children and young adults and the diagnosis is usually delayed for several weeks [2]. Tubercular involvement of the rib occurs from direct extension from nearby pleuro-pulmonary foci or by hematogenous spread from distant foci [6]. Usually the skeletal tuberculosis is associated with a primary focus in the lung [3] but in our case, we were not able to detect any lesion in the lung. Faure et al [7] noted that most solitary rib involvement was most frequent located at the rib shaft (60%) as was evident in our case.
The presenting symptoms of rib tuberculosis are a painful lesion or non-tender chest wall mass or chest pain. A draining sinus has been seen in 25% of cases, but this was usually a late finding [3].

Differential diagnosis includes the metastatic or primary tumour, metabolic bone disorder or trauma [5-6].

X-ray can detect the lesion but computed tomography is considered ideal for evaluation of chest wall lesion as it shows the nature and extent of soft tissue lesion, associated intrathoracic lymphadenopathy and bone erosions [8].

Inflammatory marker and leukocyte result are often normal. Intradermal reaction is usually positive, but when negative, it does not rule out the underlying diagnosis [9-10].

Diagnosis of tuberculosis of the rib is confirmed by demonstration of granulomatous reaction on cytology and acid fast bacilli by microscopy or by culture [10-11]. Anti-tubercular drugs are the mainstays of treatment. Two months of isoniazid, rifampicin, pyrazinamide and ethambutol once daily is followed by 10 months of isoniazide and rifampicin once daily [2- 4]. Surgery may be helpful in establishing the diagnosis or treating the recurrent or complicated cases by removing the sequestrum [9-11].

Acknowledgements

No acknowledgements of financial and material support

References


CASE REPORT

NEONATAL MALARIA PRESENTING AS NEONATAL SEPSIS: A CASE REPORT

Kuan Y Lim¹, Ee L Ang¹, Kah K Tan², Sahlawati Mustakim³

1. Paediatric Department, Hospital Tengku Ampuan Rahimah Klang, Selangor, Malaysia
2. Paediatric Department, Hospital Tuanku Ja’afar Seremban, Negeri Sembilan, Malaysia
3. Pathology Department, Hospital Tengku Ampuan Rahimah Klang, Selangor, Malaysia

Abstract

Neonatal malaria may be overlooked likely due to its non-specific features and low prevalence in Malaysia. In this case report, we detail a case of neonatal malaria in an 18-day old baby girl of Myanmar origin who presented with 6 days of intermittent fever but was otherwise well. Initially, she was treated as neonatal sepsis. She then developed thrombocytopaenia and severe anaemia with persistent spikes of temperature. This prompted a series of investigations and multiple changes of antibiotics. The diagnosis of neonatal malaria surfaced when her peripheral blood film incidentally revealed the presence of \textit{Plasmodium vivax} parasites. Peripheral blood smears are simple and inexpensive. Therefore practising especially in endemic areas for malaria, we need to consider this diagnosis when dealing with neonatal sepsis that does not respond to standard treatment.

Keywords: Congenital Malaria, Neonatal Malaria, Malaria, Neonatal

Corresponding Author: Kuan Y Lim, Jabatan Pediatrik, Hospital Tengku Ampuan Rahimah, Taman Chi Lung, Jalan Langat, 41200 Klang, Selangor, Malaysia.
Tel: +6017-5245976
Fax: +603-33756190
Email: limkuanyew@gmail.com

Introduction

Based on the latest statistic by World Health Organization (WHO), 198 million cases of malaria were reported globally in 2013 with 58400 deaths reported. The disease burden is heaviest in the WHO African region in which it accounted for approximately 90% of all malarial deaths [1]. Neonatal malaria is uncommon with reported incidences varying between 0.3 to 33.0% in both endemic and non-endemic countries [2]. Most of the cases reported were from the African region. In Malaysia this entity is rarely reported.

Case Report

An 18-day old Myanmar baby girl initially presented to the local clinic with intermittent fever since day 12 of life with a documented temperature of 37.9°C. She was then referred to the neonatal unit of Hospital Tengku Ampuan Rahimah, Klang (HTAR) for presumed sepsis. She is the second child of a 30-year old mother, born term at 39
weeks gestation via normal vaginal delivery in Hospital Temenggung Seri Maharaja Tun Ibrahim, Kulai, Johor. Her birth weight was 2.9 kilogram. Birth and postnatal history were uneventful. Mother’s antenatal care was unremarkable and she denied being unwell at any time throughout the pregnancy or perinatal period. Both father and mother are Rohingya people, a group of indigenous Muslims in northern Rakhine, Myanmar who migrated to Malaysia, 6 and 3 years ago. She was residing with both parents in an oil palm estate in Skudai, Johor since birth till day 17 of life before coming to Klang to visit their relatives. Apart from the fever, baby was well and had no feeding difficulties. She passed urine and motion normally. There were no symptoms or signs that suggest respiratory or urinary tract infections. She did not have any history of ill contact. On admission she was afebrile (axillary temperature was 36.7°C) with a heart rate of 150 beats/minute and respiratory rate of 38 breaths/minute. She was pink and non-icteric. Examinations of the cardiovascular and respiratory systems were unremarkable. Abdomen was soft and there was no hepatosplenomegaly. Her initial full blood count (FBC) showed haemoglobin (Hb) 14.1 g/dL, white blood cell (WBC) 18.5 x 10^9/L, haematocrit (HCT) 42.6% and platelet (PLT) 298 x 10^9/L. Her C-reactive protein (CRP) was 11.36 mg/L.

She was first started on intravenous (IV) c-penicillin and gentamicin. Her temperature settled initially but spiked again after 48 hours, which prompted us to change IV gentamicin to IV cefotaxime. Despite changing antibiotics, she had another spike of temperature on day 7 of admission associated with rising CRP (136.05 mg/L), anaemia and thrombocytopaenia (FBC: WBC 8.29 x 10⁹/L, Hb 9.0 g/dL, PLT 93 x 10⁹/L). Antibiotic was changed to meningitic dose of IV meropenem. Initial urine culture grew a sensitive strain of Klebsiella species (repeated subsequently was negative).

In view of thrombocytopaenia and the current outbreak of dengue in Klang Valley, dengue serology was also sent. However both dengue non-structural protein-1 (NS-1) antigen and dengue immunoglobulin M (IgM) tests were negative. Her blood sample was also sent for peripheral blood film to investigate the anaemia and thrombocytopaenia.

On day 11 of admission, we noted her fever followed a cyclical pattern with spikes at every 48 hours (Figure 1) and her peripheral blood film incidentally showed malaria parasites. We proceeded with ‘Blood Film for Malaria Parasites’ (BFMP) and it revealed Plasmodium vivax (P. vivax) (Figure 2) (11461 asexual and 115 sexual per-ml of blood). The diagnosis of P. vivax malaria was further confirmed by nested polymerase chain reaction (PCR) done in the Institute for Medical Research Malaysia, (IMR). This test is highly sensitive and specific and the primer that this laboratory useds, does not share the same sequences with any other malarial parasites [3]. The test has a sensitivity of 99.55% and specificity of 80.06 % (with 95% confidence interval). The case was discussed with a Paediatric Infectious Disease consultant and she was started on IV artesunate stat, then 12 hours and every 24 hours thereafter for 6 days. She was also started on oral primaquine base daily for 2 weeks. Mother’s BFMP and PCR test were negative for any malarial parasites.
Figure 1. Temperature trend followed a tertian fever pattern with spikes at every 48 hours
Following the initiation of anti-malarial medications, her temperature settled and her platelet count improved (Table 1). However, during the course of disease her haemoglobin level decreased to 6.3g/dL on day 14 of admission, in which packed cell transfusion was required. BFMP was repeated on 3 consecutive days after completing 7 days of IV artesunate and all 3 were negative for malaria parasites. She was subsequently discharged healthy.

Table 1. Serial full blood count and CRP level for the infant. Antimalarial started on day 11 of admission

<table>
<thead>
<tr>
<th>Days since admission</th>
<th>WBC (x 10⁹/L)</th>
<th>Hb (g/dL)</th>
<th>PLT (x 10⁹/L)</th>
<th>CRP (&lt; 0.5 mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>18.5</td>
<td>14.1</td>
<td>298</td>
<td>11.86</td>
</tr>
<tr>
<td>Day 5</td>
<td>11.64</td>
<td>11.2</td>
<td>82</td>
<td>106.88</td>
</tr>
</tbody>
</table>
Neonatal malaria is described as malaria occurring within the first 28 days of life [4]. It can be acquired via blood transfusion, a bite of an infected *Anopheles* mosquito or transmitted congenitally. Congenitally acquired infection or congenital malaria is defined as the presence of asexual stages of the malarial parasites in the cord blood at the time of delivery or in the peripheral smear of the infant in the first 7 days of life [4]. One literature study has reported that the onset of symptoms occurred between 8 to 30 days [5]. In congenital malaria the parasites may be transmitted through placental passage or when the baby passes through the birth canal during labour [5,3]. The prevalence of congenital malaria varies between 0 to 23% in endemic areas [6-9]. Meanwhile the prevalence of neonatal malaria is just below 25% in a study done in Nigeria [10]. Even among those born to pregnant women with blood smears positive for malaria parasites, the incidence of congenital malaria is very low [11]. Low prevalence of malaria is postulated to be the result of protection from passive transfer of maternal antibodies and also the presence of fetal haemoglobin [4]. In addition, spontaneous clearance has also been observed in about 62% of neonates with patent parasitaemia (i.e. parasites visible on microscopy) in a multicentred study done in Nigeria [12].

Features of neonatal malaria especially *P. vivax* infection are highly non-specific. The most common presenting symptoms of neonatal malaria is fever with studies showing it as the sole presenting feature in about 77% of the cases [10, 13]. Other common features are anaemia, jaundice, pallor and hepatosplenomegaly [5]. Meanwhile features such as respiratory difficulty, convulsion, bleeding (disseminated intravascular coagulopathy), diarrhoea, vomiting and cyanosis are akin to diagnosis of severe malaria in which, most of the time are caused by *P. falciparum* [13]. To date, there are no studies reporting differences in clinical manifestations between congenital malaria and postnatally acquired neonatal malaria (either via *Anopheles* mosquitos’ bites or blood transfusion).

Owing to non-specificity of symptoms and low prevalence rate, neonatal malaria may be overlooked in low endemic areas such as Malaysia. Therefore, delay in diagnosis is not uncommon. Like most cases reported, our case was initially treated as neonatal sepsis and the patient was started on intravenous antibiotics [4, 14, 15]. The only clue that pointed towards a diagnosis of malaria is the cyclical fever pattern (Figure 1). Her fever followed a tertian fever pattern that is common in *P. vivax* infection [16]. Despite not being treated with antimalarials during the first 10 days of admission, she did not show other symptoms and signs suggestive of malaria (e.g. jaundice, feeding
difficulty and hepatosplenomegaly). Although her initial urine culture grew a sensitive strain *Klebsiella* species, it is most likely due to contamination since her urinalysis was normal and her fever did not respond despite treatment with appropriate antibiotics. In addition, a concurrent urinary tract infection would have caused a constant fever but her fever followed a cyclical pattern since admission.

As an aid for further discussion, the life cycle of *P. vivax* is described according to Mendis et al [17]: Two hosts are involved; human and female *Anopheles* mosquito. During a blood meal, a malaria infected female *Anopheles* inoculates sporozoites into the human host. These sporozoites invade the hepatocytes and then either enter a dormant hypnozoite phase or mature into schizonts. These schizonts rupture and release merozoites into the blood stream. This initial replication in the liver is known as exo-erythrocytic phase. Merozoites then invade red blood cells and evolve into trophozoites. At this stage trophozoites undergo asexual multiplication and form schizonts which will perpetuate this erythrocytic phase. Some trophozoites differentiate into gametocytes that would be taken up by a female *Anopheles* mosquito when it bites the infected human host. Inside the female *Anopheles* mosquito, these gametocytes mature into either microgametes (male) or macrogametes (female) and undergo sexual reproduction which eventually form sporozoites. These sporozoites reside in the salivary gland while waiting to inoculate another human host. The usual incubation period of *P. vivax* is between 12 to 18 days but can take up to months or years because of the presence of latent hypnozoites in the liver [18]. When *P. vivax* is transmitted via blood (eg. vertical transmission, or blood transfusion) rather than by mosquito bites, no latent hypnozoite phase occurs, as it is the sporozoites that form hypnozoites in infected hepatocytes.
In our present case, it is difficult to accurately identify the mode of transmission because, as there was no peripheral blood smear in the first week of life. The fact that she presented with fever as early as day 12 of life, suggested vertical transmission. We postulated that her mother most likely had an asymptomatic infection in the form of latent hypnozoite phase. These hypnozoites then reactivated into trophozoites that crossed the placenta and inoculated the fetus. This reactivation may have been of low parasitaemia load with mild illness that was easily dismissed. This transient parasitaemia was then cleared by the mother’s own immune system thus the BFMP and PCR test for the mother did not detect any malarial parasite. With this, it seemed likely that the baby had acquired the infection congenitally. However we were unable to completely rule out that the baby might have acquired the infection via the bite of a malarial-infected Anopheles.

The World Health Organisation (WHO) recommends chloroquine as the first line treatment to clear the erythrocytic phase of *P. vivax* and 14 days of primaquine to clear the hypnozoites of the exoerythrocytic phase [20]. Although WHO outlines treatment recommendations for malaria in children and infants less than 5 kilogram, there are
none specific for neonatal or congenital malaria. The “Management Guidelines of Malaria in Malaysia 2nd edition (2013)” which was based on the WHO “Guidelines for The Treatment of Malaria 2nd edition” (2010) recommends chloroquine as the first line treatment for congenital malaria caused by *P. vivax* [2]. Chloroquine is to be given at an initial dose of 10 mg base/kg body weight followed by 5 mg/kg at 6 hours, 24 hours and 48 hours [2]. Primaquine is not required in congenital malaria because of the absence of hypnozoites [2]. In contrast, the latest WHO guidelines published in 2015 recommends chloroquine at an initial dose of 10 mg base/kg dose followed by 10 mg/kg on the second day and 5 mg/kg on the third day [20]. Reason for change in dosing regime was not specified.

However, in regions where there is chloroquine resistance *P. vivax* malaria, an artemisinin base compound is recommended instead of chloroquine [20, 21]. In our case, IV artesunate (an artemisinin base compound) and oral primaquine was used. IV artesunate was chosen over oral chloroquine because artemisinin compounds demonstrate superior defervescence and clearance of parasitaemia [22]. In addition chloroquine resistance cases have been reported in 9 countries in which two of them are Myanmar and Malaysia [21]. Primaquine was added because there was still a possibility that the infection was transmitted via malaria-infected *Anopheles* mosquito. The dose for IV artesunate is 2.4mg/kg stat, then 12 hours and every 24 hours thereafter for 6 days. As for oral primaquine, the dose is 0.5mg base/kg once daily for 14 days. The recommended dosing regimes for IV artesunate and oral primaquine are the same for both the Malaysian and WHO guidelines.

**Conclusion**

Neonatal malaria is an uncommon disease in Malaysia. It usually presents with non-specific features such as fever and poor feeding thus leading the attending clinician to misdiagnose it as neonatal sepsis and start antibiotic at the initial point of contact. Therefore, awareness and high index of suspicion among doctors working closely with neonates is particularly important in cases of unresolved fever to avoid delay in accurate diagnosis and treatment.

**Acknowledgement**

Adelle Soo.
Michelle Soo.
Yap Fook Choy.
Jacelyn Ong.

**References**


Neonatal Malaria Presenting As Neonatal Sepsis: A Case Report


CASE REPORT

LYMPHANGIOMA OF AN UNUSUAL SITE: A CASE REPORT

Javid Jahanbakhsh, Win Mar Salmah, Norzila Tenot Abubakar, Hadif Samsudin

Department of Radiology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Malaysia

Abstract

Lymphangiomas are hamartomatous congenital malformations of the lymphatic system that usually involve subcutaneous tissues of cervico-facial region. Rarely, it can be found in subcutaneous tissue of proximal extremities, the buttocks and the trunk. Magnetic Resonance Imaging (MRI) is the best modality to assess the tumor specification and extension. We report a case of lymphangioma at a rare site with its radiological features and patient responded to the sclerosant therapy.

Keywords: Lymphangioma, Benign Vascular Abnormality, OK-432, Children

Corresponding Author: Ahmad Hadif Zaidin Samsudin, Department of Radiology, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia
Tel: +609-7676750
Email: hadif@usm.my

Introduction

Lymphangiomas are hamartomatous congenital malformations of the lymphatic system that usually involve subcutaneous soft tissues. They are rare and accounting for 4% of all vascular tumors and approximately 5.6% of all benign vascular tumors in children [1]. Near 80% of lymphangiomas developed at cervico-facial region followed by the proximal extremities, the buttocks and the trunk. Less commonly, they can be seen in the intestines, the pancreas and the mesentery. We report a case of lymphangioma occurring at a rare site which is at the buttock, with literature review on lymphangioma.

Case Report

A one-year-old girl presented with history of progressive left buttock swelling for 3 months duration. There was no tenderness or skin discoloration. On clinical examination, there was an isolated cystic swelling at the left gluteal region. It was soft in nature, did not attach to underlying bone and without any signs of inflammation. On ultrasound examination, there were elongated anechoic tubular structures in the subcutaneous tissue and they were fully compressible. No color Doppler flow was detected within the lesions (Figure 1). The findings of tubular structures without Doppler signals favor for lymphatic vessels.
Magnetic Resonance Imaging (MRI) was done at the age of 16 months and showed a lobulated, multicystic lesion located superficially within the subcutaneous fat of left gluteal region. It extended inferiorly to the proximal upper third of the thigh and measures 5.5cm x 3.5cm (width x craniocaudal) at the largest area (Figure 4(a)). The lesion was hypointense to muscle on T1-weighted image (T1WI), heterogeneously hyperintense on T2-weighted image (T2WI) and peripherally enhanced after gadolinium administration (Figure 2).
Figure 2. The first MRI performed before treatment. Axial image on TI (a), T2 (b) and post-contrast (c). Multiple cystic structures with septal contrast enhancement.
The diagnosis of lymphangioma was made and the patient was advised for aspiration and sclerotherapy. Patient underwent sclerotherapy at the age of 2 years and 5 months old. On aspiration, chylous fluid was obtained, confirming the diagnosis.

Sclerotherapy (Figure 3) was given with Picibanil (OK-432) for three times at 8 weeks and 17 weeks intervals from the last treatment. MRI was repeated at the age of 3-year-old, that is 1 year after the last therapy which showed reduction in size (Fig.4 (b)).

Figure 3. Left buttock lymphangioma sclerotherapy procedure with OK-432
Figure 4. MRI images before and after therapy. (a) Coronal T2 images before therapy. (b, c) Coronal and axial T2 image of the same lesion after therapy showing reduced in overall size.
Discussion

Lymphangiomas are developmental malformations of the lymphatic system, which is the network of vessels responsible for transporting fluid away from tissues before returning it to the blood. Lymphangiomas are benign multilobular cystic masses lined by endothelial cells. They occur as a result of maldevelopment and obstruction of the lymphatic system. Eventually, there is no communication between sequestrated lymphatic tissue and normal lymphatic system [2].

Lymphangioma can occur anywhere in the developing lymphatic system but mostly, about 75% of the cases are located in posterior cervical triangle [1]. About 20% of cases is located at the axilla and uncommonly, cases of lymphangioma has been seen in the chest wall, retroperitoneum, abdominal organs, groin and bone. The lesions size is variable and margins can be either well-defined to ill-defined. They tend to diffuse with unclear borders. The majority of lymphangioma are presented at birth and the rest usually manifest before the age of 2 years. They can appear suddenly in childhood and occasionally in adolescence or adulthood [3].

The overlying skin is often normal or a bluish hue. Lymphangioma in extremities may associate with diffuse or localized swelling or gigantism due to soft tissue and skeletal overgrowth. There was a rare type of spongiform lymphangioma in the lower extremity with a large proximal cystic lymphatic reservoir in the groin [4]. Our patient did not have skin discoloration, bone asymmetry or bone over growth.

Lymphangiomas are classified into three subtypes based on microscopic features, which are capillary and cavernous lymphangiomas and cystic hygromas [5]. Our case was a cavernous lymphangioma, which composed of large dilated lymphatic channels. This type of lymphangiomas characteristically invades surrounding tissues.

Ultrasound is the usual preliminary study, which will reveal subcutaneous soft tissue mass with tubular structures. The absence of color Doppler signals implies that the lesion is arising from lymphatic vessels and is not pulsatile like arterial system. This ultrasound feature is useful to differentiate lymphangioma from vascular hemangioma.

MRI is the best imaging modality for lymphangioma. The lesion will have variable signal intensity on T1WI and showed hyperintense on T2WI due to high water content. They can have multiple cystic lesions. Large cysts may have fluid-fluid levels due to protein or blood components. However, fluid-fluid level was not seen in our case suggestive of absences of the components. Contrast administration usually gives rim enhancement around large cysts, within septa and intervening soft tissue as seen in our case.

Teratoma and infantile fibrosarcoma are two differential diagnoses that can appear as cystic on radiologic imaging and be confused with cystic lymphangioma.

Other diagnostic imaging that can be performed are conventional and Magnetic Resonance Lymphangiography (MRL). However, both are rarely performed nowadays except for conventional lymphangiography, which still has a roll to determine the precise location of lymphatic or chylous leakage in a patient with a thoracic lymphatic anomaly [4].

Although 10% of lymphangioma will
resolved spontaneously [6], most of lymphangioma cases need treatment due to painful swelling, recurrent cellulitis or for cosmetic reasons [7]. There are two main treatments for lymphatic anomalies, which are interventional treatment of sclerotherapy and surgical resection. Due to the nature of lymphangioma growth, which is infiltrative in nature, only two thirds of lymphatic malformations can be completely excised. Neural and vascular structures must be carefully dissected to achieve a good outcome [4, 8]. The recurrence rate is 40% after incomplete excision and 17% after macroscopically complete excision. Regrowth and reexpansion from microcystic channels are responsible for postoperative recurrence [4].

For sclerosing therapy, cystic lesion should be large enough to permit injection of sclerosant. Pure ethanol, sodium tetradecyl sulfate and doxycycline were used as sclerosing agents previously, which have been replaced with newer sclerosant agent such as bleomycin and OK-432. We used OK-432 as sclerosant agent for our case. OK-432 is produced by incubation and interaction of Streptococcus pyogenes with penicillin G potassium [9]. Three cycles of injection of OK-432 into the largest cysts were done in our patient (Figure 2). The interval of 1st and 2nd treatment was about 9 weeks and for 2nd and 3rd treatment was about 28 weeks. Significant reduction in the size of the lesion has been noted after 3 sclerosant therapy session which was about 57.8% in reduction (from 5.55cm x 3.53cm to 3.21cm x 2.57cm). There are number of case reports and series that show total lesion shrinkage after OK-432 administration and some of the authors have proposed OK-432 as the first line treatment for lymphangiomas [10]. Currently, our patient is planned for another OK-432 sclerosant therapy session.

There are other types of treatment such as cryotherapy or diathermy. However it shows marginal success and may exacerbate infection. Aspirations of fluid from a larger cyst only provide temporary decompression and rarely yield a positive culture. It is no longer recommended except for urgent decompression at a specific site [8]. Radiation treatment has no benefit in the treatment of lymphatic malformations and it will cause significant morbidity in the growing child [8].

Depends on the site of the lesion, patients can present with complication such as respiratory obstruction, feeding and speech difficulties, skeletal overgrowth and maxillary malocclusion [11]. In cervical lymphangioma, the incidence of infection can occur up to 71% of the cases and most of the cases would need parenteral antibiotic administration. Ulceration can also occur due to pressure necrosis, which would be managed by dressing and course of antibiotic [11].

Conclusion

Lymphangiomas are benign vascular lesions that are commonly occur at the head and neck region, however it may occur anywhere in the body. Radiological procedure can assist in the diagnosis as well as management of the lesions.

References


Lymphangioma Of An Unusual Site: A Case Report


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CASE REPORT

INTRACRANIAL METASTATIC NEUROBLASTOMA MIMICKING SUBDURAL EMPYEMA: AN INTERESTING CASE

Nor Fazliza Hairuddin, Ahmad Tarmizi Musa, Mohd Shafie Abdullah

Department of Radiology, Hospital Universiti Sains Malaysia, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kota Bharu, Kelantan, Malaysia

Abstract

Neuroblastoma is usually presented with abdominal distension. However, central nervous system manifestations of neuroblastoma are uncommon. In this case report, patient presented with uncommon presentation of neuroblastoma and the diagnostic dilemma.

Keywords: Neuroblastoma, Central Nervous System, Metastasis

Corresponding Author: Assoc. Prof. Dr. Mohd Shafie Abdullah, Department of Radiology, Hospital Universiti Sains Malaysia, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kota Bharu, Kelantan, Malaysia
Tel: +609767 6728/6996
Email: drshafie@usm.my

Introduction

Neuroblastoma is the third most common malignancy in children after leukemia and primary CNS brain tumors. It accounts for 10–15% of childhood malignancies [1]. The primary tumor commonly arises in the adrenal gland or along the sympathetic chain, usually in the abdomen.

Metastases are present in up to 70% of patients with neuroblastoma at the time of diagnosis [1]. Secondary craniocerebral neuroblastoma manifests most often as osseous metastases involving the calvarium, orbit, or skull base. Metastatic CNS neuroblastoma may also occur anywhere in the CNS as a parenchymal, intraventricular, or spinal cord mass.

Case Report

A 2-year old boy presented with 3 weeks of high grade fever history associated with the loss of appetite and lethargy. He was treated with 2 courses of antibiotics. However, symptoms are not improving. In the ward, he developed bilateral orbital swelling. Computed tomography (CT) brain and Magnetic resonance imaging (MRI) brain was performed and diagnosed as subdural empyema. Later, he was referred to neurosurgical team and craniotomy was done. Histopathological examination (HPE) of extradural tissue reveals malignant small round blue cells tumour with differentials of PNET WHO grade 4 or metastatic malignant round blue cells tumour. This most likely represented metastatic malignant round blue cells tumour.
CT scan of thorax, abdomen and pelvis was performed to look for primary site and for staging. Currently patient is still undergoing chemotherapy.

**Figure 1.** Contrast-enhanced CT brain shows enhancing nodular lesion in subdural region with area of hypodensities. The visualized brain parenchyma is normal

**Figure 2.** Contrasted MRI brain also shows similar lesion as in CECT brain. The lesion is enhancing with area of hypointensities

Based on this CT brain and MRI brain, he was treated as subdural empyema.
Figure 3. Contrast-enhanced CT thorax and abdomen shows enhancing soft tissue mass along the paravertebral thoracic region and abdomen with area of calcification

Figure 4. Contrast-enhanced CT brain at the level of orbits shows involvement of bilateral orbit
Discussion

Patient usually presented with abdominal distension. Metastases of neuroblastoma are often seen during recurrence of disease. However, up to 60% of cases were present during the time of diagnosis [2]. CNS metastasis account for 14% of metastasis after skeleton, regional lymph nodes and liver respectively [2]. CNS metastasis of neuroblastoma can occur in the calvarium, orbit, dura, meningeal and brain parenchyma. Bony metastasis of neuroblastoma is characterized by ill defined lytic lesion with mottling appearance [3]. Extension of epidural deposits along the suture can cause erosion of the suture thus causing suture diasthesis [3]. Neuroblastoma has a predilection to metastasis to the dura, usually on the external surface over the convexities of the skull. Dural metastasis can be a brain barrier for direct invasion of intraparenchyma. Brain parenchyma involvements are extremely rare and usually occur in disseminated disease [4]. Patients can also present with periorbital soft tissue hematomas as the initial manifestation of CNS involvement of neuroblastoma [4]. CNS involvement in patients with neuroblastoma is associated with poor prognosis. Early detection and aggressive treatment may allow some patients to live longer.

As in this patient, he was initially diagnosed as dural empyema as clinically suggestive and the brain CT scan and MRI also showed subdural collection. However, the diagnosis was changed after the dural biopsy was done which later revealed that the dura lesion was the metastatic lesion of neuroblastoma. The neuroblastoma was confirmed in the primary site along the sympathetic chain of paraspinal region involving the thoracic and upper abdomen region.

This case report highlighted the uncommon presentation of neuroblastoma with diagnostic dilemma during the initial presentation of patient to hospital. Neuroblastoma has diverse manifestation including mimicking central nervous system...
diseases. This disease must always be considered in children with unexplained neurologic presentation as it will lead to early diagnosis and early initiation of treatment.

References


