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REVIEW ARTICLE

FACTORS AFFECTING HEALTH-RELATED QUALITY OF LIFE AMONG PAEDIATRIC PATIENTS WITH THALASSEMIA: A REVIEW OF LITERATURE

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Abstract

Thalassaemia is a hereditary blood disorder that is becoming a major health problem all over the world. This chronic illness harms the quality of life of the sufferers by interrupting their physical activities, school performance and social life. Hence, this review takes aim to assess the factors affecting the quality of life of thalassaemia among paediatrics patients. A comprehensive electronic search was conducted by using PubMed, Google Scholar and Science Direct. The search was limited to those articles written in English language and by using Pediatrics Quality of Life Inventory (PedsQL™) 4.0 generic core scale questionnaire only. This review notifies emerging knowledge regarding the factors affecting the quality of life among thalassaemia patients and its implications in the essential core domains for paediatrics health-related quality of life measurements: physical, emotional, social and school functioning. It also empowers a better understanding regarding thalassaemia and assists as a foundation for the development of the effective preventive strategies for it.

Keywords: Thalassaemia, Paediatrics, Factors, Quality of life, Review

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Introduction

Thalassaemia is a genetic blood disorder where it is portrayed as incomplete or no production of one or more globin chains [1]. It is an acute life-limiting, and potentially life-threatening health problem covered all over the Mediterranean zone, the Middle
East, the Indian subcontinent and Asian origins as well as in Malaysia [2].

In Malaysia itself, thalassaemia can be seen mainly among Malay and Chinese population [3]. Almost 4.5% of Malaysians population are beta-thalassaemia carriers [4] and up to 40% are HbE carriers [5]. Malaysian Thalassaemia Registry as of September 2011 indicated a total of 5,115 registered thalassaemia patients and of these, 2,207 have beta-thalassaemia major, and 1,594 have HbE-beta thalassaemia [6]. The Ministry of Health of Malaysia expected that each year, between 150 to 350 babies are born with thalassaemia [7].

Malaysian thalassaemia patients have free access to blood transfusions and chelation agents in the form of subcutaneous deferoxamine and oral deferiprone in government hospitals all over the country. Starting from the year 2012, the newer oral chelator, deferasirox was accessible especially for the younger patients. However, most of the patients still obtained the blood transfusions and iron chelation in the form of subcutaneous deferoxamine.

Children with thalassaemia appear well at birth. However, due to the incomplete or absence of adult haemoglobin, anaemia is tended to develop and then becomes progressively worse. If thalassaemia patients do not receive an appropriate treatment, it can end with an early death [8]. On the other hand, for those children that do survive, this problem has serious implications towards their health-related quality of life (HRQoL) and may disturb their education and social activities. Children with thalassaemia need to go through blood transfusions and they also have to obtain desferal injections for iron chelation therapy to eradicate excessive iron in the blood, which resulted from the blood transfusions [8, 9].

The impact of beta-thalassaemia and its serious complications linked with physical and psychosocial health problems. Beta-thalassaemia gives an effect on the children’s physical health such as physical deformity, growth restriction and delayed puberty. Children's physical health is also impacted by complications such as cardiac failure and arrhythmia, liver disease and endocrine disorders [10]. Thalassaemia also affects the children’s psychosocial health represented by emotional, social and school functioning [11-15].

As there are limited studies focused on HRQoL of thalassaemia among paediatrics patients, thus this current study brings forward to review the factors affecting the quality of life among thalassaemia children.

**Methods**

A comprehensive electronic search was conducted by using PubMed, Google Scholar and Science Direct. The search was limited to those articles written in English language and by using Pediatrics Quality of Life Inventory (PedsQL™) 4.0 generic core scale questionnaire only.

The 23-item PedsQL 4.0 encompasses the essential core domains for paediatrics HRQoL measurement: Physical Functioning, Emotional Functioning, Social Functioning and School Functioning [16]. The PedsQL requires the thalassaemia patients to recall the frequency of their problems occurred in the past month. Individual response options of which 5-point Likert scale from 0 (never a problem) to 4 (almost always a problem) was used for all items [16]. All items were then reverse-
scored and linearly transformed into 0 to 100 point scale [16].

The Physical Health Summary Score is corresponding to the Physical Functioning scale score where it assesses the changes in physical activities performed by individuals day to day. The Psychosocial Health Summary Score is the mean score result from the sum of items divided by the number of items completed in the Emotional, Social and School Functioning Subscales.

The emotional functioning measures the satisfaction, achievement of personal goals, personal controls, social interaction, self-concept and self-esteem [17] while the social functioning evaluates the existence of social relationship and activities [18, 19]. School functioning was used to assesses how many time a child absent from school due to illness and admitted to the hospital [20]. The Total Score is the sum of all the items over the number of items answered on all the scales. The Total Score indicates an overall measure of HRQoL, with higher points reflects the higher quality of life [21].

Results

After reviewing the titles and abstracts, a total of sixteen articles related to the factors affecting the quality of life of thalassaemia among paediatrics patients were identified. Of these, fourteen articles were on cross-sectional studies and the remaining articles were unmentioned designs (Table 1).

Table 1. Summary of studies included in review

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Study Design and Sample Size</th>
<th>Factors Affecting Quality of Life</th>
<th>Factors Not Affecting Quality of Life</th>
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<tbody>
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<td>Sazlina et al (2015)</td>
<td>Cross-sectional study, 70 Malaysian thalassaemia patients</td>
<td>Age, Ethnicity, Educational level, Duration of thalassaemia, Types of thalassaemia, Presence of side effect from iron chelation treatment</td>
<td>Gender, Family income, Number of siblings with thalassaemia, Marital consanguinity, Frequency of blood transfusion and iron chelation treatment</td>
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<tr>
<td>M Ismail et al (2013)</td>
<td>Cross-sectional study, 75 Malaysian thalassaemia patients</td>
<td>Pre-transfusion haemoglobin level, Frequency of iron chelation treatment</td>
<td>Age, Ethnicity, Educational level, Types of thalassaemia, Types of chelation treatment</td>
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<tr>
<td>A Ismail et al (2006)</td>
<td>Cross-sectional study, 78 Malaysian thalassaemia patients</td>
<td>Age</td>
<td>Gender, Ethnicity, Family income</td>
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<tr>
<td>Thavorncharoen sap et al (2010)</td>
<td>Cross-sectional study, 315 Thailand thalassaemia patients</td>
<td>Age at onset of anaemia before two years, Age at first transfusion before four years, Received blood</td>
<td>Gender, Frequency of blood transfusion, Types of thalassaemia, Complications, Serum</td>
</tr>
<tr>
<td>Study Authors</td>
<td>Study Type, No. of Patients</td>
<td>Factors</td>
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<td>Surapolchai et al (2010)</td>
<td>Cross-sectional, 75 Thailand thalassaemia patients</td>
<td>Family income, Type of payment, Severe condition, Age at diagnosis, Received blood transfusion and iron chelation treatment</td>
<td>Age, Gender, Educational level</td>
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<td>Torcharus et al (2011)</td>
<td>Cross-sectional, 49 Thailand thalassaemia patients</td>
<td>Unmentioned result</td>
<td>Age, Severe condition, Pre-transfusion haematocrit level</td>
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<td>Sultana et al (2016)</td>
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<td>Age, Gender, Frequency of blood transfusion</td>
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<td>Sharma et al (2016)</td>
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<td>Dhirar et al (2016)</td>
<td>Cross-sectional, 241 Indian thalassaemia children</td>
<td>Comorbid, Frequency of blood transfusion, Duration of treatment, Number of concomitant medications, Total number of hospital visits per year</td>
<td>Unmentioned result</td>
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<tr>
<td>Saha et al (2015)</td>
<td>Cross-sectional, 365 Indian thalassaemia patients</td>
<td>Gender, Socio-economic status, Illiterate parents, Marital consanguinity, Family history of thalassaemia, Types of thalassaemia, Received blood transfusion, Pre-transfusion of Hb level</td>
<td>Unmentioned result</td>
</tr>
<tr>
<td>Jafari-Shakib et al (2017)</td>
<td>Cross-sectional, 31 Iranian and Italian thalassaemia patients</td>
<td>Unmentioned result</td>
<td>Gender, Serum ferritin level, Baseline Hb level</td>
</tr>
<tr>
<td>Caocci et al (2012)</td>
<td>Cross-sectional, 60 Middle Eastern thalassaemia patients</td>
<td>Delayed start of iron chelation therapy</td>
<td>Gender, Age at diagnosis, Age at first transfusion, Frequency of transfusion, Complications, Serum ferritin level</td>
</tr>
<tr>
<td>Garaibeh et al</td>
<td>Cross-sectional, 128</td>
<td>Complications, Family</td>
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Several studies have reported age and gender as significant factors affecting the quality of life among children with thalassaemia [10, 15, 22-25]. Studies also found that ethnicity and educational level affect the quality of life among children with thalassaemia. It was reported in a cross-sectional study over one year conducted in Selangor, Malaysia [10]. However, another two studies in Malaysia did not document the same findings [11, 12]. Patients’ body mass index also does not give an effect towards the quality of life [26].

Worst total summary scores were found among thalassaemia children who had illiterate parents and their parents who married consanguinely [24]. Family income also had a strong association with the quality of life among thalassaemia patients [24, 27]. However, both Malaysian studies revealed that family income seems not to have much effect towards the quality of life [11, 12].

Children who had family history of thalassemia both or either in maternal or paternal side had significantly better quality of life scores [24]. A study in Jordan indicated the family history of thalassemia affects the quality of life [23]. Presence of comorbidity among thalassaemia children had poorer total quality of life scores as compared to those who did not [22, 28]. They also found that more number of visits to the hospital, the poorer the quality of life scores. Type of payment where the patients were covered by Universal Health Coverage Scheme was also associated with quality of life [27].

A study conducted among Middle Eastern children with beta thalassemia found that age at diagnosis affects the quality of life [29]. However, inconsistent with this finding, a Thailand study reported that age at diagnosis did not give an effect towards the quality of life among thalassaemia children [27]. Type of thalassaemia had significant result towards the quality of life [24, 30]. However, two studies did not report the same findings [12, 15]. Duration of thalassaemia was found to be significantly related to HRQoL impairment [10, 22].

Regarding the clinical and treatment characteristics, age at onset of anaemia before two years [15, 22] and age at first transfusion before four years [15] were found to be significantly associated with HRQoL with low total summary scores. Types of treatment (blood transfusion and chelation treatment) had strong association with quality of thalassaemia patients’ life [10].
Also, thalassaemia patients who received the blood transfusion in previous past months were significantly low HRQoL scores [15, 24, 27]. For the frequency of blood transfusion and its duration of transfusion therapy, it had significant association with quality of life of thalassaemia patients [13, 24].

Thalassaemia patients who were received iron chelation therapy showed low quality of life score compared to who did not [22, 27]. The frequency of iron chelation treatment was found to be a factor that affects the quality of life, but its type of chelation treatment such as Desferrioxamine, Deferasirox, Deferiprone was not associated with quality of life [12]. Moreover, Caocci et al (2012) found that delayed start of iron chelation therapy and presence of side effect from chelation treatment such as pain at injection site, nausea and vomiting had an impact on quality of life among children with thalassaemia [10].

Besides, a Malaysian study documented that pre-transfusion of haemoglobin (Hb) level was a significant predictor of HRQoL scores [12]. The lower baseline of Hb affect the quality of life [27]. This study exposed that patients with Hb level higher than 9g/dL had significantly higher total summary scores than those patients with Hb level less than 9g/dL. Serum ferritin level did not show any association with quality of life among thalassaemia patients [15, 29, 31]. However, this was inconsistent with Boonchooduang and colleagues study.

Patients with severe condition (patients who age at onset of anaemia before two years old and age at first transfusion before four years old, pre-transfusion of Hb level less than 7mg/dL and diagnosed with homozygous beta thalassaemia) was significantly associated with low HRQoL in both Thailand studies [15, 27]. Assessment of the components of HRQoL showed patients having disease complication was the factor influencing the quality of life [23] but not in study by Thavorncharoen sap et al (2010) and Caocci et al (2012).

### Physical Health Summary Score

#### Physical Functioning

The studies in this review demonstrated links between quality of life and the physical functioning among thalassaemia children. Age of thalassaemia children, gender and socio-economic status were associated with physical functioning [25]. Children who had family history of thalassaemia either on maternal or paternal side had strong association towards physical functioning [23]. Besides, patients who covered by Universal Health Coverage Scheme was significantly associated with physical functioning [27].

Types of thalassaemia was associated with physical functioning [32]. In addition, age at onset of anaemia before two years and age at first transfusion before four years were significantly related to the physical functioning in a study conducted in Thailand [15] and India [22]. The physical functioning was also significantly associated with types of treatment [10] where thalassaemia patients who received regular blood transfusion during previous three months [15] and received iron chelation treatment [27] showed significant results. However, this finding was inconsistent with a study reported by M Ismail and colleagues (2013) where they found patients who were not on blood transfusion or iron chelation treatment were a predictor of poor physical health of health-related quality of life. Surapolchai et al (2010) and Dhirar et al...
(2016) documented the frequency of blood transfusion gave an impact towards physical functioning.

Furthermore, the presence of side effects from chelation treatment also identified as factors that affecting lower physical functioning of thalassaemia patients [10]. Pre-transfusion of Hb level and patients who had low baseline Hb had significantly low HRQoL in physical dimension [12, 27]. Based on clinical characteristics, patients with early age at diagnosis before two years [27] and had disease complications [23] indicated significant results. Dhirar et al (2016) found treatment duration, number of concomitant medicines and presence of comorbidities were significantly associated with self-reported physical summary scores.

Out of all factors that were affecting the physical summary score, it was found that physical health summary score was significantly impacted by the mean annual serum ferritin level where the higher the serum ferritin level, the lower the physical quality of life score [30].

**Psychosocial Health Summary Score**

Regarding the socio-demographic details of thalassaemia patients, age was found to be related to psychosocial health summary score [15, 22]. A cross-sectional study found that patients with low family income or covered by Universal Health Coverage Scheme had significantly affected the quality of life than those children who did not [27]. While the psychological summary score was significantly result by the type of thalassaemia; the psychosocial quality of life in thalassemia intermediate was better than thalassemia major patients, and it was associated with diabetes where the presence of diabetes will worsen the HRQoL [30]. Duration of thalassaemia less than ten years impact the quality of life [10] same goes to pre-transfusion of Hb level where it significantly related to psychosocial health summary scores [12, 15]. Based on clinical characteristics, Surapolchai and colleagues (2010) study indicated that patients with severe condition, low baseline Hb, early age at diagnosis before two years and receive regular transfusion had significantly low HRQoL. The frequency of blood transfusion and iron chelation treatment also found to be significant factors that impact psychosocial health summary score [12]. Furthermore, psychosocial domain scores strongly associated with duration of treatment and number of visits to the hospital per year [22].

**Emotional Functioning**

Not all studies reported the significant factors affecting the quality of life of each emotional, social and school functioning among thalassaemia patients. The majority of the authors keep combined all of this functioning in one dimension which was psychosocial health summary scores since this three functioning act as domains in psychosocial health dimension. However, among all of the articles in this review, only one study reported the significant factors that affect emotional functioning among thalassaemia patients where they found emotional functioning was significantly associated with age, education level, duration of thalassaemia and types of treatment [10].

**Social Functioning**

There were significant association between social functioning with age, ethnicity, education level, duration of thalassaemia and types of treatment [10]. As in the
Facators Affecting Health-Related Quality Of Life Among Paediatric Patients With Thalassemia: A Review Of Literature

previous study, Thavorncharoensap et al (2010) also found that age as a significant factor that affects lower social functioning. They also found pre-transfusion of Hb level, age at onset of anaemia before two years and age at first transfusion before four years, having a severe condition and received iron chelation treatment were significantly impaired HRQoL in school functioning subscale [15].

School Functioning

Based on a study among Malaysian children, school functioning was only associated with frequency of blood transfusion [10]. Age, pre-transfusion of Hb level, age at onset of anaemia before two years and age at first transfusion before four years and having a severe condition were significantly affect HRQoL [15]. Additionally, disease complications also associated with low quality of life [23].

Discussion

Most of the studies in this current review found that the total summary scale, physical health summary scores and psychosocial health summary scores were almost similar [10-12, 15, 23, 27, 29, 31-33]. The average of total scores in Malaysian studies approximately 69.0 [10, 11, 26]. These scores were lower when compared to both Thailand studies where the average scores were between 75.0 and 79.0 [15, 27].

The difference in the scores of HRQoL domains can be seen because most of the participants in Malaysian studies were homozygous beta-thalassaemia and transfusion dependent as compared to those participants in Thailand studies. Moreover, possible reasons were revealed that there were differences in cultures, experiences and perspectives between Thailand and Malaysia. Another study reported that the mean HRQoL score in adolescents was greater than 70, which indicated good HRQoL whereby they perceived themselves as being physically active and capable to perform normal regular activities [34].

Assessment on the subscales of psychosocial health summary score found that in most of the studies, the domain of school functioning scored the lowest except in a study by Jafari-Shakib et al (2017). Thalassaemia children need to undergo blood transfusions once every three to four weeks at day care centre or hospitals. Frequent school absenteeism for hospitals visits, coping up with the school work and lack of energy when carry out academic activities may be additional problems, which affect the quality of life. However, this result was contrasted with other results in Egypt study where they found school functioning scores were better as the patients who need to transfuse their blood attended the clinic on Saturdays that were the schools’ day off [35].

The emotional dimension also affected because thalassaemia patients felt different from their peers. They attend to express negative beliefs about their lives and sometimes, they may felt sad, angry and hurt toward their illness. Children might have psychological and emotional troubles as early as the toddler stage. Some of them seek the ability to fulfil their needs on their own, however, their caregivers did not let them perform these tasks because of their illness. Thus, thalassaemia patients might develop shame and doubt about their ability to handle problems. Thalassaemia children, as they grew older, they were becoming more aware of themselves. Some of them become more responsible towards
themselves and their illness. They were becoming more rational to share and cooperate, but thalassaemia prevented them from being more productive, and a sense of inferiority might develop instead and lower their emotional domain scores [15]. Furthermore, the treatment was emotionally demanding, where the blood transfusion and chelation therapy required repeated invasive procedures and hospital visits [36].

The low HRQoL in the physical domain in Jordan study documented that their thalassaemia children reported the presence of pain, aches and low energy when performing day to day activities [23]. Living with thalassaemia illness since early childhood causes many difficulties result in activity intolerance such as fatigue, general weakness and difficulty in breathing, which resulted from low Hb levels. Another reason probably because many patients did not come regularly for blood transfusion and thus, they would suffer from anaemic consequences that poorly impacted their physical activities and limited their exercise capacity.

Thalassemia children reported that they have no problems in socialising since the majority of the studies in this current review showed higher score compared to other domains. They had no troubles in getting along with other kids or being a member of a play team. This may be due to the non-appearance of evident disease complications at a young age, resulting in decreased feeling of stigmatisation [35].

**Total Summary Scores**

This review found that age was a significant predictor of HRQoL among thalassemia patients. Adolescent patients had significantly higher HRQoL than their younger counterparts where they experienced fewer symptoms of depression, reflecting a process of adjustment and coping with their illness [13].

Furthermore, complications and type of thalassaemia were not related to HRQoL reported in Thailand study. A small number of patients having complications in their study could explain this situation. Although patients who diagnosed with homozygous beta-thalassemia were predictable to have significantly low HRQoL since they were transfusion-dependent, but non-significant relationship was found. Similarly, a small number of homozygous beta thalassemia patients possibly explained this condition.

Presence of comorbidity among thalassaemia children had poorer total quality of life scores as compared to those who did not. Comorbidities affect severely both the physical and psychological quality of life where its absence enhances the overall quality of life. The number of comorbidities was a strong predictor of the poor physical and total quality of life score [22]. Frequent visits to the hospital have a negative impact on children's lives regarding the physical burden, psychological burden and school attendance, thus affecting their quality of life. Children have a constant stress of travelling all the way from long distances and being subjected to painful investigations and transfusion procedures.

Consistent with the findings from several studies, thalassaemia patients who received the blood transfusion and patients who received frequent blood transfusion having lower health score when compared with patients receiving less frequent. The patients typically have to undergo for blood transfusions once a month depends on the severity of the illness. The attendance for an
entire day at the hospital leads to school absenteeism and might impaired HRQoL indirectly. In contrast, there were a few studies reported no significant relationship between the frequency of blood transfusion and HRQoL. This non-significant finding could be because of the questions used to assess HRQoL of the patients were related to the feelings and conditions of the patients during the previous month; therefore the number of transfusions per year might not be relevant to the HRQoL score.

This review also showed that frequency of iron chelation treatment was significantly related to HRQoL impairment. A frequent chelation therapy may lead to an increment in iron losses which indirectly influence their quality of life. In addition, delayed start of iron chelation also had an impact on total PedsQL. Delayed iron chelation can lead to excessive accumulation of iron in body organs. Health issues arise especially when excess iron is stored in the heart, liver and pancreas [29].

Additionally, pre-transfusion Hb level was found to be significant in HRQoL. The study revealed that patients with Hb level higher than 9 g/dL had a significantly higher HRQoL than those with Hb level less than 9 g/dL. This could explain by the fact that low Hb level linked with several symptoms, such as fatigue, general weakness and decreased mental alertness, which might impaired HRQoL of the patients [37]. Pre-transfusion Hb level should be supervised routinely to retain an optimal level of 9 to 10.5 g/dL [38]. In the case of blood transfusion, one possible explanation for the significant relationship between receiving a blood transfusion and low HRQoL is that patients who received blood transfusions during the three months prior to HRQoL assessment were those with low pre-transfusion Hb levels.

Baseline Hb levels did not show any association with quality of life of thalassaemia patients. Appropriate control of the disease in the patients may be the reason for almost acceptable quality of life where more than half of thalassaemia patients had Hb level greater than 8g/dL. Small sample size also could be the reason for this finding. Serum ferritin level also does not show any association with the quality of life of the patients. This inconsistent result possibly because of damage, the long-term iron overload occurs gradually; so short-term iron overload, as represented by an elevated serum ferritin level, did not cause significant visible symptoms or complications, and hence had no impact on their HRQoL.

**Physical Health Summary Scores**

**Physical Functioning**

The physical health summary score is based on the physical functioning, which assessed the level of physical activity and energy level of the participants’ over previous months. Most of the patients in the studies were transfusion dependent, and they would experience symptoms of anaemia such as fatigue and weakness few days before their presentation to their clinic’s follow-up for the frequent blood transfusion. Thus, this might affect negatively their physical functioning.

Combined oral and subcutaneous iron-chelation therapy was negatively predicted the physical health, due to the burden of nightly subcutaneous injections of desferrioxamine and daily oral deferiprone tablet. Severe cases where the patients with transfusion dependence and iron overload
probably need combined iron-chelation therapy to achieve serum ferritin levels below 1,000 to 1,500 ng/mL [39], a threshold that is known to be the most reachable tool and related with a reduced risk of iron overload-related complications such as heart failure in patients with thalassemia.

Presence of side effects from chelation treatment also affects physical health of thalassaemia patients. The burden of subcutaneous injections of iron chelation treatment five to seven days a week was associated with impaired HRQoL [15]. Furthermore, it is known that among the common side effect of chelation treatment are the pain at the injection site, nausea and vomiting. This side effect may contribute to the reduced scores in this study as most of the patients received five or more times in a week of chelation treatment [27].

Since the severity of thalassaemia and treatment were not always associated with reductions in the HRQoL, a Thailand study by Surapolchai et al (2010) is the first report presenting a significant relationship between the HRQoL and family financial impact in thalassaemia patients. Surprisingly, type of payment had an impact on both children and parents’ perspectives. Self-payment could be implied to be higher household income and affordable medical expense; which assisted to predict the better parent-rated HRQoL.

**Psychosocial Health Summary Scores**

Predictor of lower psychosocial health summary score was the duration of thalassaemia less than ten years [10]. In the present study, those with duration of thalassaemia were those patients aged less than 13 years old. The younger patients would have fewer experiences in dealing with their illness and may have trouble to understand the disease they have, which could affect them emotionally. Moreover, in this study, most of the Malaysian children aged less than 13 years were on blood transfusion. Consequently, their social and school functions also affected as they need to be away from school to receive the transfusion or any other treatment. Hence, this could affect their psychosocial health. The older children could have more knowledge about thalassaemia, and they were able to understand the support from their parents, carers or peers to allow them to cope better.

Regarding the emotional functioning, adolescent males were found to have better emotional dimension than adolescent females [34]. Peer influence can be the reason for poor emotional functioning in female adolescents. Bullying and teasing due to the physical appearance probably affect the individuals’ self-esteem resulting in poorer HRQoL [40]. Sociocultural pressures such as family influences may also directly have an impact on the HRQoL of the adolescent's girls resulting in poor overall HRQoL [40].

When looking at subdomains of psychosocial health dimension, patients who received the blood transfusion and iron chelation treatment affect school functioning. Thalassaemia children need to require the blood transfusion to meet body demands. Literature reviews discovered that patients who received the blood transfusion related with school functioning dimension. It may be due to multiple visits to hospitals where these patients need to transfuse their blood. For several patients, maybe they had same complications after they get the blood transfusion. So in that case, they need to admit into the ward for more days.
Conclusion

Age, severe types of thalassemia, received of blood transfusion, serum ferritin level, frequency and side effects of iron chelating treatment, and presence of complications are the most documented contributory factors for low quality of life in thalassemia patients. This review gives an emerging knowledge regarding contributory factors that has a big impact in clinical practice in order to improve thalassemia patients’ quality of life. This serves as a foundation for the development of an effective preventive strategy via screening of thalassemia carrier among pre-marital adults as well as school students.

Acknowledgement

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Factors Affecting Health-Related Quality Of Life Among Paediatric Patients With Thalassemia: A Review Of Literature


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Factors Affecting Health-Related Quality Of Life Among Paediatric Patients With Thalassemia: A Review Of Literature


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CRISPR/Cas9: An Introduction To Genome Editing

REVIEW ARTICLE

CRISPR/CAS9: AN INTRODUCTION TO GENOME EDITING

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Abstract

Application of genome editing have garnered a lot of attention in the scientific world. Its ability to make permanent and precise modification or ‘edits’ in the genomic DNA sequences have opened up the possibility of probing the functionality of genes and correcting abnormal genes to treat genetic diseases. The CRISPR/Cas9 which was adapted from Type II bacterial adaptive immune system is the most applied genome editing system due to the ease and efficiency of the system in customising the nucleases needed in editing the targeted sequences. However, the downside of this system is the high frequency of ‘off-target’ editing caused by the nature of the Cas9 protein that can tolerate mismatches between customised nucleases; sgRNA, and the genomic sequences. Despite the set-back, researchers continue applying CRISPR/Cas9 system to edit the genome by minimising the frequency of ‘off-target’ edits. Application of the improved system had successfully produced numerous results in the initial treatment of genetic diseases such as cystic fibrosis, β-thalassemia, and Duchenne Muscular Dystrophy. The fast-paced development and improvement made to this system will continue to be utilised by the researchers around the world for the treatment of human diseases, a progress much needed and awaited.

Keywords: CRISPR/Cas9, Genome Editing

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Introduction

For the past decade, we had witness a surge of genome editing application due to their ability to inactivate genes/insert intact genes and correct mutated sequences by either point mutations or precise edits at desired loci in the genome. Introduction of targeted modifications in the genome to treat genetic diseases, probe the function of a gene or other genomic feature by altering the existing DNA patterns with great precision, requires extremely efficient system [1, 2].

Recently developed genome editing technologies using viral, cellular and synthetic nucleotide templates [3] are guided by engineered site-specific nucleases or
"molecular scissors". The most common engineered nucleases are meganucleases derived from microbial genetic elements, zinc finger nucleases (ZFNs) of eukaryotic transcription factors, transcription activator-like effector nucleases (TALENs) from Xanthomonas bacteria, and also clustered regularly interspaced short palindromic repeats associated RNA guided Cas9 (CRISPR-Cas9) nucleases which is adapted from Type II bacterial adaptive immune system [4, 5].

Engineering programmable sequence-specific DNA-binding nucleases such as ZFNs and TALENs to enable genome editing are time-consuming and very challenging, significantly impeding the usage of these techniques. On the other hand, the ease and speed of designing high efficiency site-specific CRISPR-guided nucleases, and the possibility for multiplexed editing had driven the application of CRISPR/Cas9 system to be the most widely used genome editing tool in different organisms for various biological applications [6, 7]. A simple comparison of TALENs, ZFNs and CRISPR/Cas9 systems adapted for genome editing are summarised in Table 1.

All the nucleases are engineered to introduce double strand breaks (DSBs) at the desired target sites, a crucial step that will triggers the cellular DNA repair pathways; the error-prone non-homologous end joining (NHEJ) and homology directed repair (HDR) to introduce targeted modifications in the genome or ‘edits’ [8, 9]. With advances and improvements done to increase the specificity and minimize ‘off-target’ effect, genome editing can be used to permanently alter genomic sequences by targeted disruption such as insertion or excision in both in vivo and ex vivo settings [8], thus opening up the possibility to a more comprehensive treatment and cure of genetic diseases such as β-thalassemia, Huntington disease, cystic fibrosis and X-linked chronic granulomatous [10].

In this review, we focused on the basic mechanism of CRISPR/Cas9 system, advances made to improve the design and flexibility of the system to minimized ‘off-target’ editing and its clinical application.

<table>
<thead>
<tr>
<th>Table 1. Comparison between ZFNs, TALENs and CRISPR/Cas9 Genome Editing Systems</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>System</strong></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Basic feature</strong></td>
</tr>
<tr>
<td><strong>Target site interaction</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Genome alteration</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Design</td>
</tr>
<tr>
<td>System efficiency</td>
</tr>
<tr>
<td>Off target effects</td>
</tr>
<tr>
<td>Multiplexing capability</td>
</tr>
</tbody>
</table>

Zinc Finger Nucleases – ZFNs  
Transcription Activator–Like Effector Nucleases – TALENs  
gRNA – guard RNA (tracrRNA+crRNA)
Cas9 - CRISPR-associated protein-9 nuclease
NHEJ - Non-homologous end joining
HDR - Homology directed repair

**Overview of CRISPR/Cas9 System**

CRISPR, a genome editing technology derived from a bacterial innate immune system found in variety of bacteria and archaea, are fragments of prokaryotic DNA containing short repetitions of base sequences [11, 12]. Three different types of CRISPR/Cas system had been identified; Type I, II and III that utilises different mechanism in generating CRISPR RNA (crRNA) and Cas proteins [13].

For the purpose of genome editing, researchers adapted the Type II system (Figure 1) based on its simplicity in requiring only CRISPR-associated protein 9 (Cas9) protein from *Streptococcus pyogenes* and two other RNA components, crRNA and trans-activating crRNA (tracrRNA), that fused to become guide RNA (gRNA) [1]. In this system, the tracrRNA which is complementary to the repeat sequences in pre-crRNA triggers processing by double-stranded RNA-specific ribonuclease, RNase III in the presence of Cas9 protein [14]. The gRNA then binds to Cas9 protein and direct the newly formed complex to a target sequence in the genome based on complementary base-pairing rule [1] that allows a stretch of ~18 to 20 nucleotides of the gRNA sequence to hybridize with the targeted sequence, docking the Cas9 nuclease at that location [15].

Cas9 that consist of two different domains; HNH and RuvC-like, requires a base-pairing structure forming between the activating tracrRNA and targeting crRNA (gRNA) to cleave the double stranded DNA (dsDNA) [16]. Locus of site-specific cleavage in the genome is determined by base-pair mediated binding to complementary DNA sequences between crRNA (of the gRNA structure) and the target sequence. The target sequence must be adjacent to a protospacer adjacent motif (PAM), a short motif consisting of ‘NGG’ or ‘NAG’ sequence (N is either A, T, C or G) [1, 16].

The dsDNA strand complementary to the target-binding crRNA sequence is cleaved by Cas9 HNH domain while the non-complementary DNA strand is cleaved by Cas9 RuvC-like domain at a site three base pairs upstream of the PAM [15, 16]. The DSB generated by Cas9 will induce the cell to repair the damage by either NHEJ or HDR. This is the focus point where researchers can manipulate the CRISPR/Cas9 system to modify the genome at targeted locations [1, 17, 18] (Figure 2).

NHEJ; in the absence of exogenous donor DNA, involves highly efficient but error-prone direct ligation of DNA ends that causes small insertions and/or deletions known as ‘InDels’ at the off-target DSB sites, either of which could alter protein coding sequences and induce termination of protein expression [19]. In disease-causing locus, NHEJ can be manipulated to disrupt (knock-out), excise, or restore open reading frame (ORF) of gene of interest. Meanwhile, activation of HDR requires a donor DNA carrying DNA sequences homologous to those adjacent to the DSB at the targeted locus to initiate homologous recombination between the donor and targeted sequences. The donor DNA can be used to repair a mutation by “knocking-in” point mutation at the targeted genomic locus to replace the mutated sequence, or insert exons or full cDNA at endogenous locus to replace the
abnormal gene at a genomic “safe harbour” where it will be integrated and expressed without random insertional mutagenesis and alteration of gene function [8, 9, 20].

Adaptation of CRISPR/Cas9 system for genome editing in eukaryotes could be carried out in three different strategies: the single guide RNA (sgRNA) which is an artificially constructed gRNA, and Cas9 protein can be expressed as i) DNA, ii) RNA or iii) RNA/protein complexes. In the first strategy, expression of the components by DNA will involves the introduction of two plasmids DNA encoding for sgRNA and Cas9 protein respectively. The DNA sequences for crRNA: tracrRNA hybrid and Cas9 will be transcribed in vivo into sgRNA and Cas9 protein [21, 22]. The second strategy involves mRNAs encoding for sgRNA and Cas9, which is used to lessen the in vivo burden by dispensing with the plasmid transformations and DNA transcription step [23]. Alternatively, the last strategy involves the use of purified Cas9 protein which have been complexed with the sgRNA which could be introduced into the cell as RNA/protein complex [24], simplifying the introduction of the component by eliminating the need to optimize gene expression in vivo to establish the initial system.
Figure 1. Targeted genome editing mediated by RNA-guided Cas9
Figure 2  Basic mechanism of double strand break DNA repair by Non-homologous End Joining (NHEJ) and Homology Directed Repair (HDR)
Minimizing CRISPR/Cas9 ‘Off-target’ Effect

sgRNA and Cas9 protein are the main components required for CRISPR/Cas9 genome editing system [25]. Custom designed sgRNA is simplified to a chimeric functional structure consisting of a variable region (crRNA) composed of 18 to 20 nucleotides complementary to the targeted genomic DNA sequence, followed by a PAM motif and a basic scaffold (tracrRNA) used to bind Cas9 protein to form a gRNA/Cas9 complex [25, 26].

The downside of this CRISPR/Cas9 system, Cas9 protein which act as a molecular scissors, can tolerate mismatches between sgRNA and the targeted genomic sequence to a certain extent (three to five base pairs), in the PAM-distal part of the sgRNA-guiding sequence that resulted in ‘off target’ effects, as some previous studies have shown [1, 27]. ‘Off-target’ in genome editing refers to unintended genetic modifications caused by lack of specificity between engineered nucleases and the targeted sequences. The DNA mismatches at the 5′ end of sgRNA are better tolerated than those at the 3′ end; known as the “seed sequence”, which determined the specificity of Cas9 [7, 26, 28]. Any mismatches in PAM sequence as well as the 8 to 12-base of the seed sequences located at the 3′-end will eliminate targeting [25, 27]. Studies showed that optimizing the sgRNA design by emphasising on both seed sequence and PAM-distal target sequences, which possibly triggers a conformational change of Cas9 [29], can influence the efficiency of the Cas9 binding and lower ‘off-target’ editing compared to only optimising the seed regions [1, 26].

Various bioinformatics tools have been developed by applying predictive modelling approaches to identify optimal gRNAs, such as ‘on-target’ prediction software, Azimuth (https://crispr.ml/) [30] that directs Cas9 protein to targeted DNA sequence and ‘off-target’ prediction software, Elevation (https://crispr.ml/) [31] that identify potential ‘off target’ loci to help reduce the ‘off-target’ editing probability in the genome with the application of CRISPR/Cas9 system. These predictive software computationally search and determine genomic sequences with high sequence similarity to the targeted locus by first-order sequence comparison [5].

Activity of Cas9 protein is another factor that can cause ‘off target’ editing with methods of Cas9 delivery into cells [32] and elevated levels of the protein [33, 34] being associated with unspecific DNA sequence cleavage. Hence, efforts to control the Cas9 activity by limiting the dosage of active protein; enough to modify targeted sequence but insufficient to cleave potential off-target sequences; may lead to substantial reduction of unwanted consequences [1, 35]. Several approaches that use chemical and physical agents such as 4-hydroxytamoxifen (4-HT) [35] and tetracycline/doxycycline (Tet/Dox) [36] as a temporal control, limits the activity and exposure time for Cas9 protein in the genome, thus reducing the ‘off-target’ effect.

Improved specificity that results in reduced ‘off-target’ editing has been reported by the used of direct delivery of purified Cas9 protein and sgRNA into cells compared with plasmid DNA transfection encoding for Cas9 and sgRNA. Plasmid transfection is often inefficient and stressful to cells [26, 32]. The cleavage of chromosomal DNA occurs immediately after delivery of Cas9-sgRNA ribonucleoprotein (RNP) complexes into the cells before the complexes are degraded rapidly, reducing the time and
amount of active Cas9 protein in the genome, thus decreasing the ‘off-target’ effect. In contrast, Cas9 protein expressed from transfected DNA plasmids may not reach maximal levels within 24 hours, thus allowing more time for ‘off-target’ lesions to develop [32].

However, focus on the frequency of ‘off-target’ sites and their locations are not sufficient to ensure the safety of CRISPR/Cas9 genome editing system. Subsequent biochemistry/bioinformatics/molecular analyses can reliably predict function, as not all deletions or insertions are benign; to identify and evaluate cellular toxicity caused by transformation of cells produced by the ‘off-target’ editing [2].

For therapeutic application, since genome editing leads to permanent modifications in the human genome, the frequencies of genomic ‘off-target’ mutation must be kept at absolute minimum, preferably at ‘zero off-target’. To effectively address the broad spectrum of genetic disorder and achieve the much-needed level of therapeutic efficacy in human, CRISPR/Cas9 system must be able to generate highly efficient homologous recombination in the target specific diseased tissue without any unwanted modification elsewhere [5].

**Application of CRISPR/Cas9 in Clinical Research**

CRISPR/Cas9 system had been widely used as research tools for genome editing application in human diseases with customised programmable nucleases enabling precise and permanent gene correction at the DNA level [37]. The ability to manipulate the genome by perturbation of genomic DNA has opened up opportunities to treat genetic disorders especially monogenic disorder such as β-thalassaemia, sickle cell anaemia and cystic fibrosis by correcting the causative mutation [5].

Development of CRISPR/Cas9 and other genome editing systems is a significant improvement for gene therapy treatment of genetic diseases. In 2013, the CRISPR/Cas system was first used for effective correction of disease-related genes in intestinal stem cells of a cystic fibrosis (CF) [38]. In 2013, Schwank and colleagues [38] had cultured intestinal stem cells from a cystic fibrosis patient homozygous for fibrosis transmembrane conductance regulator (CFTR) mutations. CRISPR/Cas-mediated homologous recombination was applied to correct the targeted CFTR locus. The corrected allele was successfully corrected and confirmed to be functional.

Apart from that, β-thalassaemia is one of the most common genetic diseases caused by mutations in the adult β-globin (HBB) gene. Xie and colleagues had efficiently corrected mutations of the HBB gene in patient-derived induced pluripotent stem cells (iPSCs) by using CRISPR/Cas9 combined with piggyBac transposon. The corrected iPSc were differentiated into erythroblasts with restored expression of HBB, retaining pluripotency and exhibiting normal karyotypes without any ‘off-target’ effect reported [39].

Kaminski’s research group [40, 41] employed an RNA-guided CRISPR/Cas9 DNA editing system to accurately remove the entire HIV-1 genome; a crucial gene required for HIV integration in host genome, between 5′ and 3′ long terminal repeat (LTRs) of integrated HIV-1 pro-viral DNA copies from latently infected human CD4+ T-cell and delivered the CRISPR/Cas9 system by a tail vein injection. The treated animals exhibited a reduced expression of
the targeted HIV gene in multiple tissue organs, alluding viral infectivity reduction induced by CRISPR/Cas9 genome editing in vivo. A brief summary of some of diseased-related research applying CRISPR/Cas9 genome editing system are summarized in Table 2.

Table 2. Summary of CRISPR/Cas9 Utilized in Clinical Translation Studies

<table>
<thead>
<tr>
<th>Disease</th>
<th>Target location</th>
<th>Cells type</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic granulomatous disease</td>
<td>CYBB gene</td>
<td>iPSCs from skin fibroblast</td>
<td>Restoration of oxidative burst function in iPSCs-derived phagocytes</td>
<td>[42]</td>
</tr>
<tr>
<td>β-thalassaemia</td>
<td>HBB gene</td>
<td>iPS from fibroblast</td>
<td>Recovery of HBB expression in iPSCs</td>
<td>[43]</td>
</tr>
<tr>
<td>β-thalassaemia</td>
<td>HBB gene</td>
<td>iPS from skin fibroblast</td>
<td>Recovery of HBB expression in iPSCs</td>
<td>[39]</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>CTFR gene</td>
<td>3D-intestinal organ cultures (organoids)</td>
<td>Patient’s organoids showed functional recovery</td>
<td>[38]</td>
</tr>
<tr>
<td>Haemophilia A</td>
<td>F8 gene</td>
<td>iPSCs from urine-derived cells</td>
<td>iPSCs showed functional recovery</td>
<td>[44]</td>
</tr>
<tr>
<td>Duchenne muscular dystrophy</td>
<td>Dystrophin gene</td>
<td>iPSCs from fibroblast</td>
<td>iPSCs showed functional recovery</td>
<td>[45]</td>
</tr>
</tbody>
</table>

iPSCs - induced pluripotent stem cells
Conclusion

The past few years have seen a spurt of CRISPR/Cas9 genome editing application of which have been rapidly developed and applied in numerous diseases. The flexibility, ease and efficiency of this editing system have open possibilities in genome manipulation. The capability to use the same platform in gene knock-out and knock-in to be utilized in basic biological and clinical translational studies will lessen the complications and challenges for the researchers. The ability of CRISPR/Cas9 with the help of donor DNA provide a good strategy to enable a corrective genomic DNA modification in situ in zygotes or patients diagnosed with childhood inherited genetic diseases such as β-thalassaemia, Duchenne Muscular Diseases and cystic fibrosis. For now, monogenic diseases are the most amenable for CRISPR/Cas9 genome editing since only a single allele needs to be targeted and corrected to cure the diseases.

However, several challenges must be addressed, especially those relating to efficacy of the genome editing and safety concerns of the system related to the Cas9-associated ‘off-target’ effects in therapeutic applications. Delivery efficiency of genome editing components into the targeted cells and tissues must also be considered to make sure the system is run at their maximum potentials. Despite the challenges, with improved knowledge on programmable nucleases and DNA repair mechanism and with better understanding on disease pathology, success of therapeutic genome editing especially in monogenic diseases will be much anticipated.

References


PARENTING STRESS IN MALAYSIAN PARENTS OF CHILDREN WITH THALASSAEMIA

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Abstract

Introduction: Parents of children with chronic illness such as thalassaemia, experience parental anxiety as a result of multiple stressors; these parents will consequently undergo the process of coping and adjustment in order to overcome the situation. Identifying families who may require psychosocial intervention is an important step towards providing holistic management for these children.

Methodology: A cross-sectional study was conducted to determine the level of parenting stress amongst parents of children with thalassaemia. Risk factors associated with high parenting stress score were identified. Research instruments used were the Parenting Stress Index/Short Form and Coping Inventory for Stressful Situations.

Results: A total of 54 subjects were recruited. High total parenting stress score (TPSS, defined at 75th centile) for this cohort was 99.5 whereas the mean TPSS was 86.18. Overall, 24% of these parents have high TPSS. Chinese ethnicity, number of children, monthly income, task-oriented and emotion-oriented coping mechanisms were significantly associated with a high TPSS. Logistic regression analysis revealed that low task-oriented but high emotion-oriented scores were risk factors with the greatest predictive power.

Conclusion: Early recognition of parenting stress is important to identify at risk parents so that interventions such as counseling and social support may be given.

Keywords: Parenting Stress, Thalassaemia, Total Parenting Stress Score

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Introduction

Having a child with a chronic illness has a major impact on the family as a whole. Parents of children with chronic disease experience difficulties in dealing with feelings which may subsequently lead to a dysfunctional family [1,2]. Parenting stress is the pressure experienced by parents caused by their interactions with their
The sources of distress vary; these include disease severity, treatment procedures, uncertainty of treatment outcome and financial burden.

Thalassaemia is a common single gene disorder requiring lifelong treatment. In Malaysia, it has been estimated that between 150 to 350 babies are born with thalassaemia each year [4]. Although life expectancy has significantly increased, various multi-system complications that are either disease or treatment related, have now come into focus [5,6]. Patients with thalassaemia major also have significantly disturbed school functioning and maladjustment [7]. Mazzone et al (2009) have shown that there is significant impairment in mothers of children with thalassaemia, in terms of physical and psychological health, and overall quality of life [8].

Coping strategies encompass both cognitive and behavioral attempts to manage internal and external demands that are considered taxing or exceed one’s resources [9]. Based on the interactive model of stress, anxiety and coping as postulated by Endler (1990), interaction of “person variables” (such as trait anxiety) with “situation variables” (such as life events) may lead to certain reactions which results in a changed state of anxiety [10,11]. This may lead to a feedback loop and becomes a continuous process. Thus, parents who have a child with chronic illness will undergo the process of coping and adjustment to a stressful situation. If parents cannot adapt, handle the situation, or deal appropriately with their children, they lose confidence, which will then affect their parenting skills as well [12]. This will ultimately affect the management, compliance to treatment and future outcome of these children. The purpose of this study was to determine the level of parenting stress amongst parents of children with thalassaemia and to identify risk factors including coping strategies that are associated with high parenting stress score.

Materials and Methods

This cross-sectional study was conducted from November 2008 till June 2009 at the Paediatric Haematology/Oncology Clinic and Thalassaemia Day Care Centre, Universiti Kebangsaan Malaysia Medical Centre (UKMMC). Parents of children with thalassaemia intermedia or thalassaemia major, age 12 years and below, were recruited and written informed consent was obtained. Universal sampling was used. Both parents were given a set of self-administered questionnaire. An additional set of questionnaires were given (for completion at home) if only one parent was present upon recruitment. These questionnaires were returned on the same day or on the subsequent clinic appointment. The questionnaires comprised two research instruments: the Parenting Stress Index/Short Form (PSI/SF) and Coping Inventory for Stressful Situation (CISS); the questionnaires which were available in both English and Bahasa Malaysia have been established and validated in a previous research that was conducted locally [11-14].

The PSI/SF consists of 36 items questionnaire, with statements on a five point Likert scale and is a direct derivative of the full-length PSI-120 through a series of replicated factor analyses [12]. It evaluates three domains: parental distress, parent-child dysfunctional interaction and difficult child. Total parenting stress score (TPSS) is obtained by adding the scores of the three domains together; it is an indication of the overall level of parenting stress. Test-retest reliability and α reliability for the PSI/SF total scores were 0.84 and 0.91 respectively.
The total score for PSI/SF is divided into centile range. In our study, high TPSS is defined as a total score of more than the 75th percentile.

The CISS is a 48-item self-administered questionnaire with statements on a five point Likert scale ranging from ‘never’ to ‘very often’ [11]. The items are divided into 3 subscales: task-oriented, emotion-oriented and avoidance-oriented. Task-oriented coping focuses on purposeful efforts taken to solve a problem, restructure a task, or modify a situation. Emotion-oriented coping describes efforts used to reduce stress by emotional reactions, personal preoccupations or daydreaming; whereas avoidance-oriented coping focuses on activities or cognitive modifications that are used to avoid stressful situations [11].

Statistical analysis was performed using the SPSS programme version 16.0. Independent student t-test was used to assess differences in reported PSI/SF and CISS scores between mothers and fathers. Independent student t-test and chi square test were used to assess the difference between variables in the high TPSS and low TPSS; if the quantitative variable was not normally distributed, the Mann-Whitney U test was used. Following bivariate analysis, logistic regression analysis was performed to identify variables that were significantly associated with a high TPSS.

### Results

A total of 54 parents participated in this study, of whom 20 are fathers and 34 are mothers. The majority of the respondents were of Malay ethnicity. Table 1 summarised the sociodemographic characteristics of the parents.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in years (SD)</td>
<td>39.16 (7.08)</td>
<td>2000</td>
</tr>
<tr>
<td>Median Monthly Income, RM</td>
<td>(IQR: Q1:1000; Q3: 3665)</td>
<td></td>
</tr>
<tr>
<td>Relation to patient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>20 (37%)</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>34 (63%)</td>
<td></td>
</tr>
<tr>
<td>Ethnic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malay</td>
<td>42 (78%)</td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>12 (22%)</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>49 (91%)</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>5 (9%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Socio-demographic characteristics of parents of children with thalassaemia (N=54)
Parenting Stress In Malaysian Parents Of Children With Thalassaemia
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IQR: Interquartile Range

Cut-off score for high TPSS in our study was 99.5. Mothers had a higher mean TPSS and mean score on all three domains of PSI as compared to the fathers (Table 2). However, only the difference in mean score for the “Difficult Child” (DC) domain was statistically significant ($p<0.05$).

Table 2. Comparison of mean scores of the domains in the PSI between mothers and fathers of children with thalassaemia

<table>
<thead>
<tr>
<th>PSI</th>
<th>MOTHER mean (SD)</th>
<th>FATHER mean (SD)</th>
<th>$p^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPSS</td>
<td>89.26 (21.32)</td>
<td>80.95 (24.31)</td>
<td>0.19</td>
</tr>
<tr>
<td>PD</td>
<td>30.85 (8.16)</td>
<td>27.15 (8.51)</td>
<td>0.11</td>
</tr>
<tr>
<td>PCDI</td>
<td>27.50 (7.90)</td>
<td>26.60 (9.23)</td>
<td>0.70</td>
</tr>
<tr>
<td>DC</td>
<td>30.91 (7.80)</td>
<td>27.20 (8.98)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Independent t-test; PSI: Parenting Stress Index; TPSS: Total Parenting Stress Score; PD: Parental Distress; PCDI: Parent-Child Dysfunctional Interaction; DC: Difficult Child

Table 3 illustrates the comparison in mean scores of coping mechanisms between mothers and fathers. The task-oriented mean scores for fathers were significantly higher than mothers ($p<0.05$). On the other hand, even though the mean score for maternal emotion-oriented coping mechanism was higher when compared to fathers, it was not statistically significant.

Table 3. Comparison of mean scores of the coping mechanisms in CISS between mothers and fathers of children with thalassaemia

<table>
<thead>
<tr>
<th>CISS</th>
<th>MOTHER mean (SD)</th>
<th>FATHER mean (SD)</th>
<th>$p^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Task Orientated</td>
<td>58.52 (8.40)</td>
<td>64.70 (10.81)</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Analyses of risk factors associated with a high TPSS were performed. These factors were categorized into child and parental characteristics and parental coping mechanisms. Based on bivariate analysis, there were no statistically significant on child-related risk factors: these include age, gender, clinical phenotype (thalassaemia intermedia or major), use of chelation therapy, duration since diagnosis and other similarly affected siblings. As for parental-related risk factors, parental ethnicity and number of children within the family were significantly associated with a high TPSS (Table 4). There were seven parents who have only one child. Five of these seven parents have TPSS score of more than 99.5 (i.e. 103, 105, 108, 110 and 131), with a mean score of 100.71, while the remaining two parents have TPSS of less or equal than 99.5. In the group of parents who had more than one child, only eight of the remaining 47 parents had a TPSS score of more than 99.5, with mean score of 84.02 ($p=0.06$). The mean income in the high TPSS group was significantly lower as compared to those from the low TPSS group.

Table 4. Predictive factors for high TPSS: Parental characteristics

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>HIGH TPSS (n = 13)</th>
<th>LOW TPSS (n = 41)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%) (%, unless stated otherwise)</td>
<td>(%) (%, unless stated otherwise)</td>
<td></td>
</tr>
<tr>
<td>Mean age in years (SD)</td>
<td>39.30 (8.93)</td>
<td>39.12 (6.53)</td>
<td>0.93</td>
</tr>
<tr>
<td>Mean monthly income (RM), (SD)</td>
<td>1523 (1323)</td>
<td>2585 (1709)</td>
<td>0.04*</td>
</tr>
<tr>
<td>Relation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>3 (15.0%)</td>
<td>17 (85.0%)</td>
<td>0.38</td>
</tr>
<tr>
<td>Mother</td>
<td>10 (29.4%)</td>
<td>24 (70.6%)</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>11 (22.4%)</td>
<td>38 (77.6%)</td>
<td>0.74</td>
</tr>
<tr>
<td>Divorced</td>
<td>2 (40.0%)</td>
<td>3 (60.0%)</td>
<td></td>
</tr>
<tr>
<td>Ethnic group</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Independent t-test
CISS : Coping Inventory for Stressful Situation
Based on Table 5, the group with a low TPSS had a significantly higher mean task-oriented score as compared to the group with a high TPSS ($p<0.05$). In contrast, the emotion-oriented score was significantly higher in the group with the high TPSS ($p<0.05$). Logistic regression analysis was subsequently performed following bivariate analysis. Two factors were found to be predictive i.e. task-oriented coping mechanism and emotion-oriented coping mechanism. Task-oriented score was inversely related to high TPSS (B -0.16; OR 0.85), whereas a high emotion-oriented score was significantly associated with high TPSS (B 0.11; OR 1.11).

**Table 5. A comparison CISS mean score between study groups**

<table>
<thead>
<tr>
<th>CISS</th>
<th>HIGH TPSS mean (SD)</th>
<th>LOW TPSS mean (SD)</th>
<th>$p^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Task-oriented</td>
<td>55.00 (6.74)</td>
<td>62.65 (9.88)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Emotion-oriented</td>
<td>44.92 (12.45)</td>
<td>37.39 (11.02)</td>
<td>0.04*</td>
</tr>
<tr>
<td>Avoidance-oriented</td>
<td>41.61 (10.20)</td>
<td>45.48 (11.47)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*Independent t-test; CISS: Coping Inventory for Stressful Situation; TPSS: Total Parenting Stress Score

**Discussion**

To date, there is no published study addressing the issue of parenting stress using PSI/SF amongst parents of children with thalassaeemia. From this study, the mean score for total parenting stress score (TPSS) was 86.18. In comparison to other studies amongst various chronic childhood diseases, parents of children with inborn errors of metabolism and Duchenne muscular dystrophy have a mean TPSS of 76 and 81.2 respectively [15,16]. Although the mean TPSS value of the aforementioned studies appears comparable to this study, there are still notable differences. These include the type of childhood illness, different ethnic groups with different socioeconomic
backgrounds and cultural beliefs in addition to the support systems.

Within the local context in a case control study by Syed Abdullah (2004), the mean TPSS for mothers of children with epilepsy was 97.87 as compared to 85.07 for the control group [14]. Whereas in another local study by Abdul Razak NN (2005), the mean TPSS for mothers of children with spina bifida and the control group was 92.4 and 78.2 respectively [17]. Both mean TPSS for mothers of children with epilepsy and spina bifida were higher than the mean TPSS for parents of children with thalassaemia. The difference in these scores may be attributed to the fact that children with neurological disease tend to have more behavioural problems when compared to children with thalassaemia.

Although not statistically significant, the mean maternal TPSS was higher compared to paternal TPSS i.e. 89.26 and 80.95 respectively. In a study amongst parents of children with cancer in Taiwan, Yeh et al (2002) concluded that maternal parenting stress scores were significantly higher than paternal parenting stress scores across all three domains of the PSI [18]. There are several possible reasons contributing to the higher maternal TPSS in our study. Mothers are often regarded as the main caregiver of the sick child and the other siblings. The need to balance this responsibility with other life roles (e.g. as a wife) might be perceived as an additional “burden” to these mothers. Furthermore, mothers are often more willing to admit discomfort and stress than the fathers [19]. In addition to this, one spouse may not have perceived or experienced the same responsibility or obligation of caring for the ill child than the other parent.

In our study, there was a higher refusal rate of participation amongst fathers compared to mothers. The group of fathers who refused may actually represent the group with high TPSS and may not be willing to reveal that openly. The fact that the majority of fathers did not participate in this study may also have a possible impact on the accuracy of these findings that paternal TPSS was lower than maternal TPSS. It is interesting to note that the mean task-oriented coping score for fathers was higher compared to mothers (64 and 58 respectively, p value 0.02). As males tend to adopt a problem-solving strategy when faced with a stressful situation, this may have resulted in the lower paternal TPSS [20].

Bivariate analysis showed that lower parental monthly income, Chinese ethnicity, having only one child, having a lower task but higher emotion-oriented coping score for coping mechanism, were significantly associated with having a TPSS beyond the 75th centile. The effect of ethnicity and increased level of stress was also shown by Ong et al (1998, 1999), where Chinese mothers of children with mental retardation and cerebral palsy were noted to have a higher levels of stress [21,22]. In the UK, non-white ethnic groups of parents of children with thalassaemia reported different patterns of family adaptation to having a disabled child, indicating there is cross-cultural differences in terms of how they interpret the stressors and how they adopt different believes to adapt to these situations [7]. As Malaysia is a multicultural population, health care professionals need to be aware of these differences when dealing with parents from various ethnic groups.

Cost of treatment and transportation for frequent hospital visits pose a great financial burden to those with a lower monthly income. This will increase the strain in family resources which may ultimately impair the parents’ coping mechanism and
increase their stress level. From our study, mean income in the high TPSS group was significantly lower as compared to those from the low TPSS group. This was similar to the study done by Syed Abdullah (2004) who looked at parenting stress among mothers of children with epilepsy in Malaysia [14]. Several studies have also shown that lower income and lack of paid jobs are associated with higher stress level among parents of cerebral palsy and sickle cell disease children [23-25].

From this study, having only one child was a significant factor associated with higher parenting stress level. In this particular situation we postulate that the parents may feel guilty as they have a child with a genetic disorder that is inherited from them and thus, all of the parenting attention is focused onto that one child. However, our finding differs from other studies looking at parenting stress among caregivers of children with chronic illness, as shown in a systematic review by Cousino & Hazen (2013). In this review, the number of children in a family is not associated with higher parental stress [26]. In another study by Olley et al (1997), mothers of more than one child with sickle cell disease reported greater parenting stress [27]. However, this aspect was not explored in our study.

Logistic regression analyses showed that having a lower task-oriented but higher emotion-oriented score were significantly associated with high TPSS. Task-oriented coping mechanism concentrates on resolving problems; thus, these parents have better adaptive mechanism. Emotion-oriented coping mechanism, generally seen as a maladaptive coping mechanism, was significantly associated with a high TPSS with an odds ratio of 1.11. In this coping strategy, the parents are only “releasing” the emotions that they feel in response to the stressful situation, but the problem itself is not addressed nor resolved. In the short term, it may be beneficial as the parents are relieving their emotional burden but in the long term, it is considered maladaptive because this might delay the efforts needed in planning on how the problems should be solved. These results however need to be interpreted with caution. As this is a cross-sectional study, thus a definitive conclusion that there is a vicious cycle or a chain reaction between a high level of stress and maladaptive coping mechanisms cannot be assumed. In addition, other life stressors were not assessed in greater detail. In view of this, a longitudinal study focusing on the chronological relationship of coping strategies and stress level should be carried out.

This study has several limitations; firstly, there is no established centile score for clinically significant stress level based on the PSI/SF for Malaysian population. Further studies are therefore needed to determine clinically significant stress level at or above 90th centile of stress score in the Malaysian population. The small sample size was another limitation. The actual proportion of parents who finally took part was only 54 compared to the total number that was eligible i.e. 90. This may have skewed the results as those who refused to participate may be the ones with the higher TPSS. Furthermore, as this is a cross-sectional study, collection of data is made only at one point in time. The stress level might have evolved with time, and different coping mechanisms may have been used at different phase of illness. It is important to note that increased parenting stress does not necessarily lead to dysfunctional state or poorer health-related quality of life; thus further studies are needed to determine the relationship between childhood
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thalassaemia, parenting stress and quality of life in both child and parents.

Conclusion

Twenty four percent (24%) of parents of children with thalassaemia have high level of parenting stress and mothers of these children have higher scores as compared to the fathers. Paediatricians need to be aware that parents of children with thalassaemia can be under considerable stress even though the child’s disease status appears to be clinically manageable. High parenting stress may result in psychological sequelae, thus affecting the overall care of the child. Therefore early recognition of parenting stress is important and the parents of children with thalassaemia especially at risk parents should be evaluated so that interventions such as counseling and social support may be given.

Disclaimer

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Acknowledgement

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References


COMPARISON BETWEEN YOGURT AND COMMERCIAL PROBIOTICS IN THE DURATION OF DIARRHEA IN PEDIATRIC AGE GROUP

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Abstract

Objective: To compare the mean duration of diarrhea between children taking yogurt and with the group taking commercial probiotics, containing lactobacillus in acute watery diarrhea. Materials and Methods: This descriptive study was conducted in the Department of Pediatrics, Services Hospital, Lahore. The duration of this cross sectional study was 6 months, from June 2013 to November 2013. A total of 200 children suffering from acute watery diarrhea were involved in the study after the informed consent under the said hospital. Their demographic information was recorded and later divided in two groups of 100 each; group A received yogurt and group B was given commercial probiotic. The duration of diarrhea was observed in both groups. The results were compared by using independent sample t-test. Results: The average duration of diarrhea in group A, was 1.98 + 1.31 day while that in group B was 3.09 + 1.64 days. Student's t-test was applied and the difference between the two groups was found to be statistically significant (p< 0.05). Conclusions: Treatment with yogurt significantly decreases the mean duration of diarrhea as compared to probiotic among patients with acute watery diarrhea.

Keywords: Lactobacillus, Yogurt, Probiotics, Duration of Diarrhea

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Email: atifamin@unisza.edu.my

Introduction

Diarrhea is defined as passage of 3 or more loose stools per 24 hours [1]. In developing countries diarrhea accounts for millions of deaths each year in young children. In developed countries, it is a common cause of admission in emergency departments [2].
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Diarrhea accounts for 18% of mortality under 5 years and more than 5000 deaths every day [3], 78% of deaths are from African and South-East Asian region. Pakistan contributes 500 deaths per day in this death toll [4]. According to a Pakistan Medical Association report, the incidence of diarrhea among children in Pakistan is 20% [5].

Conventional treatment modalities for acute watery diarrhea include intravenous fluids, oral rehydration solution (ORS) with Zinc supplements which reduces the duration of diarrhea by 25% and stool volume by 30%. Home remedies include rice water and yogurt [6]. Children with poor reserves are at higher risk of complications like repeated hospital admission, malnutrition, electrolyte disturbance (hypokalaemia) and prolonged hospital stay [7]. Appropriate clinical assessment of a patient should be done to guide a cost effective and evidence based approach for diagnostic investigations and treatments [8]. Oral rehydration solutions have reduced the complications caused by diarrhea to a significant extent, but it neither changes the consistency of stools nor normalizes the gut flora [9].

Probiotics are viable as well as, non-pathogenic microorganisms which when given in proper amount, offer a health benefit to the host. Examples of the commonly used bacterial probiotics include Lactobacillus species, Escherichia coli Bifidobacterium species, Streptococcus species, and the yeast Saccharomyces boulardii [9,10].

Lactobacillus bulgaricus and Streptococcus thermophilus are responsible for the fermentation of milk that results in the formation of yogurt. Commercially available yogurt usually contains Lactobacillus bulgaricus and Streptococcus thermophilus in equal proportions [15]. It can be easily prepared at home and is believed to be beneficial in diarrheal disorders in most of Pakistani families.

In Turkey, a trial of comparison of yogurt and probiotic Saccharomyces boulardii demonstrated that in both groups stool consistency normalized at the same time (3.07 ± 2.01 days versus 3.07 ± 1.73 days (P > 0.05). As far as duration of hospitalization was concerned, there was no difference among both groups (4.68 ± 2.37 versus 4.23 ± 1.72 days; P=0.45) [16]. A subgroup analysis in the same study on patients with rotavirus infection showed reduction in diarrhea duration in the group taking yogurt (4.61 ± 1.68) compared with Saccharomyces boulardii (5.47 ± 2.37) (P=0.74) [17]. A study in Italy compared the placebo with lactobacillus. After enrollment, duration of diarrhea was 3.0 ± 1.14 days in placebo versus 2.42 ± 1.15 days in lactobacillus group (mean ± SD with P value = 0.03) and in rotavirus-positive children, duration of diarrhea lasted 3.19 ± 1.73 days versus 2.34 ± 0.7 days respectively (P < 0.008). Diarrhea continued for more than 7 days in 10.7% of placebo as compared to 2.7% of lactobacillus group (P < 0.01). Hospital stay
was significantly less in group B than in group A [18]. There is no local study available comparing the yogurt (natural source of Lactobacilli) and commercial probiotics containing lactobacillus, but many trials of different strains of lactobacillus show efficacy in diarrheal diseases [19].

In Pakistan, diarrhea related morbidity and mortality are quite high in children below 5 years especially in low socioeconomic status. Little work has been done about the affect of yogurt supplementation on duration of diarrhea. Yogurt has advantages with respect to cost effectiveness, easy availability, community trust in addition to its probiotic role as well as nutritional value. The rationale of this study is to observe whether yogurt is as effective as commercially available probiotics (lactobacillus) in decreasing duration of diarrhea so that we can suggest a cheaper and easily available remedy for the treatment of a very common disease such as Acute Watery Diarrhea.

Material and Methods

The study was carried out in the Department of Pediatrics, Services Hospital, Lahore. A total of 200 cases were selected for this cross-sectional study; 100 in each group after informed consent from the parents under the hospital, with 95% confidence interval, 80% power of test and taking magnitude (mean ± SD) of duration of diarrhea with yogurt supplemented group and commercial lactobacillus probiotics group i.e 3.07 ± 2.01 and 2.42 ± 1.15, respectively. Duration of study was 6 months. Inclusion criteria were children presenting with acute watery diarrhea between 6 months to 5 years having grade IV or more watery stools greater than 3 per day for more than 2 days. Children who were simultaneously receiving antibiotics, who had clinical or lab evidence of bacillary dysenter aty and lactose intolerance, who developed complications like ileus: sepsis, acute renal failure or had history of co-morbid conditions like chronic renal failure, chronic liver disease and third degree malnutrition were excluded. Their demographic information including name, age, sex, address was recorded. 200 children fulfilling the inclusion criteria were admitted through emergency and out-patient department. Approval from Hospital ethical committee of Services Hospital, Lahore was taken. Informed consent from parents was taken and risks and benefits of study were explained. Cases were registered for the study and demographic information of the patients (name, age, sex, address) was obtained.

On receiving the patient in ER, vital signs were recorded and examination was done to assess for signs of dehydration. After preliminary rehydration and stabilization, patients were shifted to the ward and divided into two groups by lottery method. Standard treatment with oral/IV fluids and anti-pyretic (if indicated) was started to both groups. One group (A) was advised to take yogurt (natural source of lactobacillus), 20mL twice a day along with standard treatment and other group (B) to take probiotics one sachet twice a day with standard treatment. Patients were reassessed daily by asking mother for grading and frequency of stools till improvement. Less than 3 stools of grade 1 per day were considered as improvement. Duration of diarrhea from both groups was recorded on a predesigned proforma.

Data was analyzed by using SPSS-14 for windows. Qualitative data i.e. sex was presented as percentage and frequency.
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Quantitative data (age and durations of diarrhea) were presented as mean, standard deviation and duration of diarrhea were compared by using independent sample t-test. A p-value of 0.05 or less was statically significant.

Results

The total number of patients included in the study was 200 (including both males and females) and they were divided in two equal groups A and B respectively. The mean age of the patients included in group A was 22.140 ± 10.556 months (range 6 - 60 months). There were 14% patients of age range of 6 - 12 months, 16% patients of age range of 13 - 24 months, 28% patient of age range of 25 - 36 months, 22% patients of age range of 37 - 48 months, and 20% patients of age range of 49 - 60 months. (Table 1)

Table 1. Distribution of patients by age (n=200)

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Group A</th>
<th></th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>6 - 12</td>
<td>14</td>
<td>14</td>
<td>27</td>
</tr>
<tr>
<td>13 - 24</td>
<td>16</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>25 - 36</td>
<td>28</td>
<td>28</td>
<td>21</td>
</tr>
<tr>
<td>37 - 48</td>
<td>22</td>
<td>22</td>
<td>17</td>
</tr>
<tr>
<td>49 - 60</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>22.140 ± 10.556</td>
<td>21.091 ± 9.977</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.381</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>6 - 60</td>
<td>6 - 60</td>
<td></td>
</tr>
</tbody>
</table>

Patient's mean age included in group B was 21.091 ± 9.977 months [ranges from 6 - 60 months]. There were 27% patients of age range of 6 - 12 months, 15% patients of age range of 13 - 24 months, 21% patient of age range of 25 - 36 months, 17% patients of age 37 - 48 months, and 20% patients of age range of 49 - 60 months. Chi-square test was applied and there was no significant difference between the two groups (p > 0.05) (Table 1).

Patients were also distributed according to sex. There were 66% male patients in group A, while 35% patients were female. Female to male ratio was 1:1.63. In group B, there

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were 71% male patients, while 29% patients were female. Female to male ratio was 1:2.45. Chi-square test was applied and no significant difference was detected between the two groups (p > 0.301). (Figure 1)

Figure 1. Distribution of patients by sex (n=200)

The mean duration of diarrhea in group A was $1.98 \pm 1.31$ days, while that in group B was $3.09 \pm 1.64$ days. Student's t-test was applied and difference between the two groups was found to be statistically significant ($p < 0.05$). (Table 2)

Table 2. The comparison of mean duration of diarrhea in two groups (n=200)

<table>
<thead>
<tr>
<th>Mean duration of Diarrhea</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean + SD</td>
<td>$1.98 \pm 1.31$</td>
<td>$3.09 \pm 1.64$</td>
</tr>
<tr>
<td>p-value*</td>
<td>0.004**</td>
<td></td>
</tr>
</tbody>
</table>

Key:
SD  Standard deviation
*   Student’s t-test
**  Not significant

Discussion

Probiotics are useful bacteria that can help in controlling the episodes of acute watery diarrhea. This study was a comparison of a probiotic lactobacillus, and yogurt in controlling the consistency of stools and their frequency to less than 3 in 24 hours. This was one of the largest prospective, randomized controlled trials so far, comparing the two preparations in a local Pakistani population including 200 children. The results of this study were in favor of yogurt and it was found that by its use there was decrease in the episodes of diarrhea. This was one of the larger studies including 200 children suffering from acute watery diarrhea comparing the lactobacillus with
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In our study, the mean age of patients was 22.140 ± 10.556 months in group A and 21.091 ± 9.977 months in group B, while the mean age of the patients in study by Makbule et al. 2010 [17] was 21.2 ± 28.2 months. In another study by Heydarian et al. 2010 [20], the mean age of patient was 2.5 ± 2.3 years. This difference in mean age of patient is mainly due the inclusion criteria adopted by different authors. Majority of the patients were male i.e. 62% in group A, and 72% in group B. In study by Heydarian et al. 2010 [20] male patients also predominated the female patients i.e. 57% patients were male and 43% were female. In another study by Eren et al, [17] 65% were male and 35 % were female.

Van Niel, 2002 [21] in his meta-analysis, focused on randomized and double blinded studies on different strains of Lactobacilli in 122 children. Those children, who had received antibiotics recently, were excluded from the study. It was found that Lactobacilli decreased the duration of diarrhea by 0.7 days. It was also noticed that the frequency of stools was reduced to 1.6 on day 2. Simakachorn et al. 2000 [22] conducted a study on 53 children comparing the lactobacillus with yogurt and observed lesser duration of diarrhea with treatment with bacillus (1.81 ± 1.08) days and with placebo (2.38 ± 1.51). Shornikova et al. 1997 [23] conducted a study on 46 children comparing outcome of yogurt with placebo. In this study, 21 patients received treatment with yogurt and 25 were controls. They found that duration of diarrhea was shortened in patients who were treated with yogurt (1.5 ± 1.1 days) as compared to controls (2.5 ± 1.5 days).

Billoo et al. 2006 [24] evaluated the efficacy of Sachromyces boulardi in children suffering from acute watery diarrhea in Pakistan. He concluded that the duration of acute watery was reduced in children given Saccharomyces Boulardii as compared to control group (n=50, 86.4 hours versus n=50,115.2 hours, respectively; P=0.001). Stool frequency on days 3 (P=0.01) and 6 (P=0.001) was also decreased in the group receiving Saccharomyces Boulardii.

Another study was conducted by Stefeno et al. 2000 [18] to compare the placebo with lactobacillus. Duration of watery diarrhea after enrollment was 3.0 ± 1.14 days in placebo versus 2.42 ± 1.15 days in lactobacillus group (mean ± SD; P=0.03) and in rotavirus-positive children, diarrhea lasted 3.19 ± 1.73 days in placebo versus 2.34 ± 0.7 days in lactobacilli group respectively (P < 0.008). The previous studies in literature have shown more improvement with probiotics as compared to placebo. However, when the two groups, one taking probiotics and other receiving yogurt were compared, it was found that there is improvement in the diarrheal episodes in the group taking yogurt.

The diarrhea in children may be improved due to strengthening of mucosal integrity of the intestine. Probiotic strains may adhere to receptors of epithelial cells of intestinal wall and protect it from offending microorganisms. So, absorptive defects of fluids and electrolytes and glucose are prevented. Probiotics also reinforce production of secretary IgA. The T cell activity may be enhanced by probiotics and decrease in chloride secretion of epithelial
cells, causes cessation in diarrheal episodes. Probiotics may also exhibit some antimicrobial activity that can prevent offending organism from proliferation [25].

The action of probiotics takes few days to establish colonies and causing their potential effects. After reaching the optimal colonization, they reveal potential benefits like stimulation of immune system including antibodies secretion and anti-inflammatory effects.

One of the limitations of the study was that it was conducted in an area where rich patients present in our tertiary care unit. So, it may not be a true representation of actual population of the Pakistan. This was not a double blinded study. Yogurt and probiotic sachet were physically different from each other.

Conclusion

Treatment with yogurt significantly improves the diarrheal episodes as compared to those children receiving probiotics. Hence, it is concluded that yogurt should be given to children suffering from acute watery diarrhea. To the best of our knowledge this is the first report for the said objectives in Lahore, Pakistan.

References


Comparison Between Yogurt And Commercial Probiotics In The Duration Of Diarrhea In Pediatric Age Group
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ORIGINAL ARTICLE

PREVALENCE OF HEPATITIS B AND C INFECTIONS IN CHILDREN INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS IN ABAKALIKI, EBONYI STATE, SOUTHEAST, NIGERIA

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Abstract

Objective: Human immunodeficiency virus (HIV) infected children with chronic viral hepatitis infection may have increased morbidity and mortality when compared to HIV negative children. This study was aimed at determining the prevalence of hepatitis B virus (HBV) and C virus (HCV) infections among HIV infected children in Federal Teaching Hospital Abakaliki. Methods: It was a cross sectional study that involved consecutive recruitment of 88 confirmed HIV infected children aged 2-17 years, attending the antiretroviral therapy (ART) clinic. Testings of hepatitis B surface antigen and anti-HCV antibodies were done using the ACON hepatitis B and C rapid test strips (Acon laboratories Inc San Diego. CA). Results: A total of 88 subjects were recruited during the study period, prevalence of hepatitis was 4.5% (4/88). HIV/HBV co-infected was noted in 3.4% (3/88) of the subjects while HIV/HCV in 1.1% (1/88). Hepatitis B and C infections were highest among children more than 12 years of age and children from lower socio-economic class. There were however no significant relationships between hepatitis B and C infections and socio-demographic variables. There was also no significant relationship between prevalence of hepatitis B and C infections and immunologic stages of subjects. Although there was no case of hepatitis observed among HIV infected children on TDF/FTC/EFV drug combination, antiretroviral regimen had no significant relationship with prevalence of hepatitis B and C infections. Conclusion: The prevalence rates of HIV/HBV and HIV/HCV co-infections observed in this study are low when compared to previously reported prevalence rates. Sustained efforts at strengthening HBV immunization program and other preventive measures are recommended.

Keywords: ART, HBV, HCV, HIV/AIDS, Immunization, Immunodeficiency

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Background

Infection by human immunodeficiency virus (HIV) is a global phenomenon [1]. Sub-Saharan Africa is endemic for HIV and HBV infections while prevalence of HCV infection is not well documented in this region [2]. Nigeria has the largest burden of HIV in Africa, accounting for 14.0% of the total Africa’s burden and records the highest rate of MTCT of HIV infection in the region [3-4]. Studies in Nigeria revealed prevalence rates of hepatitis B surface antigen ranging from 0.5 to 44.7% [5-7]. Importantly, these three viruses (HIV, Hepatitis B and C viruses) are transmitted vertically from mother to child and also transmitted via blood and blood products [8, 9]. Major risk factors include blood transfusion, scarification marks, circumcision, indiscriminate use of sharps, surgeries [8].

Nwolisa et al. [10] reported a prevalence rate of 5.8% of HBV infection among HIV infected children in a hospital based study. In a similar study Sadoh et al. [8] reported a prevalence of HBV infection of 7.7% among 155 HIV infected children. Nworie et al. [11] in a study among HIV negative children in a rural community of Ebonyi state, reported a prevalence rate of HBV infection as 6.5%. The high prevalence rate of HBV infection in these children was attributed to poor access to immunization. Peri-natal transmission of HCV infection occurs in approximately 3-5% of infants born to women infected with HCV. HIV infection has been associated with persistent HCV viraemia. Sadoh et al. [8] noted a prevalence rate of 5.5% of HIV/HCV co-infection.

Both HBV and HCV acute infections can progress to chronic infections [12-13]. Compared with patients who are infected with HIV alone, co-infected adults are more likely to develop chronic hepatitis, to have more severe liver disease, to experience HIV treatment failure, to have higher viral loads (for both viruses), and to develop antiretroviral-associated hepatotoxicity [14]. HBV infections acquired in the perinatal period and early childhood is more likely to lead to chronic infections [12]. Similarly chronic infections are likely to result in about 60-80% of children with acute infections from HCV [13].

Study by Nworie et al. [11] was carried out among HIV negative children in Ebonyi state. There is no data on the prevalence of hepatitis B and C infections among HIV infected children in Ebonyi state. Hence there is need for this study.

Aims and Objectives

The study was aimed determining the prevalence of Hepatitis B and C infections among children confirmed to have HIV in Abakaliki, Ebonyi state. The study also aimed to determine the relationship between hepatitis (B and C) infections and immunodeficiency stage of HIV infection.

Methodology

Study area

Ebonyi State has a total population of 2,173,501 people, majority of which are Igbos [15]. The study is a cross sectional hospital based study carried out in Federal Teaching Hospital Abakaliki (FETHA), the Ebonyi State capital, from August 2015 to March 2016. The Federal Teaching Hospital Abakaliki (FETHA) operates provider-initiated HIV testing and counseling (PITC), in which every child that presents at the Children Out-patient Clinic is offered HIV
antibody test irrespective of presenting complaint, except on objection by the caregiver, however objection (opt-out) of the caregiver to the screening test does not affect quality of treatment given to the child. Any child who tested positive to the test is referred to the Paediatrics Infection Disease Clinic for further evaluation and management.

**Determination of sample size**

Sample size was calculated using the prevalence rate of hepatitis infection reported by Sadoh et al. [8] (12.8%). A minimum sample size of 88 was obtained.

**Ethical considerations**

The study was conducted as part of the study on haematological screening of HIV infected children in FETHA. Ethical approval was sought and obtained before the commencement of study. The study was explained to parents/guardian and only those who gave informed consent were included in the study.

**Subject selection**

The subjects that have been regular to Paediatric Infection Disease clinic in FETHA in the past one year prior to the study and newly diagnosed HIV infected children that were referred from the Children Outpatient Clinic within the study period were recruited consecutively until sample size was met. A structured questionnaire was used to obtain information on socio-demographic characteristics, history of blood transfusion, other risk factors and use of ART.

Blood samples that were collected for haematologic profile of subjects were screened for hepatitis B and C viruses. Testing of HBsAg and Anti-HCV ab were done using the ACON hepatitis B and C surface rapid test strip (Acon laboratories Inc San Diego, CA). These rapid tests have sensitivity of 88.8% and specificity of 100.0% [16].

**Data analysis**

The data obtained was entered into spreadsheet using the Microsoft excel 2007 and the analysis was done using the Statistical Package for Social Science version 19.0. Descriptive results were expressed as frequencies and percentages. The significance of associations between categorical variables was tested using Pearson’s chi-square and Fischer’s exact tests for comparison of proportions. The level of statistical significance was achieved if p < 0.05

**Results**

A total of 88 subjects were recruited during the study period. Majority were females 46 (52.3%). Male to female ratio was 0.91:1. The age range of participants was 2-17 years. Majority of the children were 6-12 years (62.5%) and were of lower socio-economic class (69.3%). Although prevalence rates of hepatitis B and C were commonest among subject greater than 12 years of age and lower socio-economic class, there were no significant relationships between these variables and prevalence rate of hepatitis as shown in Table 1. HCV infection was observed in a male adolescent that was of lower socio-economic class. Risk factors to acquisition of hepatitis did not influence prevalence rate of hepatitis in this study as shown in Table 1.
Among the study participants, 3 out of the 88 subjects (3.4%) were positive for Hepatitis B infection and only one out of the 88 subjects (1.1%) had hepatitis C infection giving an overall prevalence rate of hepatitis infection to be 4.5% (4/88).

Table 1. Distribution of HBV and HCV infections according to Socio-demographic characteristics of subject

<table>
<thead>
<tr>
<th>Socio-demographic</th>
<th>Number examined (%)</th>
<th>Number Positive for HBV (%)</th>
<th>Number Positive for HCV (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5</td>
<td>13 (14.8)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>6-12</td>
<td>55 (62.5)</td>
<td>1 (1.1)</td>
<td>0 (0.0)</td>
<td>0.346</td>
</tr>
<tr>
<td>&gt;12</td>
<td>20 (22.7)</td>
<td>2 (2.3)</td>
<td>1 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>42 (47.7)</td>
<td>1 (1.1)</td>
<td>1 (1.1)</td>
<td>1.893</td>
</tr>
<tr>
<td>Female</td>
<td>46 (52.3)</td>
<td>2 (2.3)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Socio-economic class</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>8 (9.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>19 (21.6)</td>
<td>1 (1.1)</td>
<td>0 (0.0)</td>
<td>0.762</td>
</tr>
<tr>
<td>Lower</td>
<td>61 (69.3)</td>
<td>2 (2.3)</td>
<td>1 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>12 (13.6)</td>
<td>1 (1.1)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Unsafe injections</td>
<td>28 (31.8)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Surgeries</td>
<td>5 (5.7)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0.602</td>
</tr>
<tr>
<td>Scarification marks</td>
<td></td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Not applicable</td>
<td>36 (40.9)</td>
<td>2 (2.3)</td>
<td>1 (1.1)</td>
<td></td>
</tr>
</tbody>
</table>

The only patient with HIV/HCV co-infection was in severe immunodeficiency stage of disease as shown in Table 2. Majority of the subjects were in ‘not significant immunodeficiency stage’ of HIV infection and this stage recorded no case of hepatitis of either hepatitis B or C. Although majority of subjects with hepatitis B infection were in advanced stage of disease, there was no significant relationship between immunodeficiency stage and prevalence rate of hepatitis (B and C) infections. This is also shown in Table 2 below.

Table 2. Relationship between Immunodeficiency and hepatitis B & C infections

<table>
<thead>
<tr>
<th>Immunodeficiency Stages</th>
<th>Positive for Hepatitis</th>
<th>Negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg (%)</td>
<td>Anti-HCV ab (%)</td>
<td></td>
</tr>
<tr>
<td>Not Significant</td>
<td>0 (0.0)</td>
<td>43 (48.9)</td>
</tr>
</tbody>
</table>
Prevalence Of Hepatitis B And C Infections In Children Infected With Human Immunodeficiency Virus In Abakaliki, Ebonyi State, Southeast, Nigeria


Table 2 shows that two out of the 15 subjects that were not on ARV (ART naïve) had hepatitis B and C infections. Majority of subjects on antiretroviral (ARV), were on first line medications. Table 3 shows that fifty (56.8%) of the children that were on AZT/3TC/NVP combination were negative for hepatitis infection while all the 12 children who were on TDF/FTC/EFV were negative for hepatitis B and C infections. Out of the 10 children on second line of drug, only one (1.1%) had hepatitis B and none with hepatitis C, as shown in Table 3.

There was no significant relationship between ARV use and prevalence of hepatitis B and C infections as shown in Table 3 below.

Table 3. Use of ARV/Type of ARV versus positive HBsAg & Anti-HCV ab

<table>
<thead>
<tr>
<th>ARV Use</th>
<th>Type of ARV</th>
<th>HBsAg</th>
<th>Anti-HCV ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>AZT/3TC/NVP</td>
<td>1 (1.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>TDF/FTC/EFV</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>ABC/3TC/LPV/r</td>
<td>1 (1.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>1 (1.1)</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>3 (3.4)</td>
<td>1 (1.1)</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 0.362, \ p = 0.834 \]

*AZT= Zidovudine, 3TC= Lamivudine, NVP= Nevirapine, TDF= Tenofovir, FTC=Emtricitabine, EFV=Efavirenz, ABC=Abacavir, LPV/r=Lopinavir/ritonavir

Discussion

HIV infection is predominant in women of reproductive age and people of lower socio-economic class [17]. This group of people may also have high prevalence of hepatitis considering their common mode of transmission. High prevalence rates of hepatitis B and C in women of reproductive age translates to high maternal to child transmission of the virus. Although hepatitis was highest among subjects in lower socio-economic class in index study, there was no significant relationship between hepatitis infections and socio-economic class. The high prevalence rate of hepatitis in children from lower socio-economic class may be a reflection of the total number of HIV infected subjects that are from lower socio-economic class (69.3%). This corroborates finding by Nworie et al. [11] and Sadoh et al. [8] who observed hepatitis most in subjects in lower socio-economic class and no formal education in caregiver.

The prevalence rate of hepatitis B infection in the index study is consistent with HBV prevalence rate of 2.6% reported by Toussi et al. [18] but lower when compared with that reported by Sadoh et al. [8] Nwolisa et
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al. [10] and Nworie et al. [11] whose prevalence rates of HBV infection were reported as 7.7%, 5.8% and 6.5% respectively. This lower prevalence rate experienced in this study may be explained by the increased awareness and accessibility of caregivers to immunization program and preventive measures. The prevalence rate of HCV infection observed in this study was consistent with the prevalence rate of 1.5% reported by Schuval et al. [19] but lower when compared to that prevalence rate of 5.5% reported by Sadoh et al. [8] This may be a reflection on the mode of transmission of the hepatitis C virus. Telatela et al. [14] observed that in settings where antenatal screenings for HCV among pregnant women showed high prevalence rate, children born to such mothers would also be positive for the infection and vice versa. Zhou et al. [9] reported a high prevalence rate of 9.6% of HCV infection among HIV infected children in China and attributed the high rate to unsafe blood transfusion. This is contrary to index study where the only case of HCV infection observed, did not have history of blood transfusion, surgery, unsafe injections and scarification marks, suggesting that the virus may have been vertically transmitted from mother. Only one out of the 12 subjects with a history of blood transfusion had HBV infection. This underscores the effectiveness of the blood screening techniques in the health facilities. This is contrary to Zhou et al. [9] that reported a significant relationship between blood transfusion and hepatitis infection.

With introduction of highly active antiretroviral therapy, there is maximal suppression of HIV replication, reduction in viral load and increased cellular immune responses to opportunistic infections such hepatitis B and C infections [20]. This may be the reason for low prevalence rates of hepatitis B and C in index study, as majority of subjects were on HAART. Combination of tenofovir (TDF) with lamuvidine (3TC)/emtricitabine (FTC) is highly recommended as a highly effective first-line treatment for HBV infection [21]. The above combination with efavirenz (EFV) is recommended treatment for adolescent with HIV/HBV co-infection [21]. It is therefore not surprising that there was no record of hepatitis B viral infection among subjects on this medication in index study.

Hepatitis was observed to be highest in children with advanced HIV/AIDS (advanced and severe immunodeficiency stages) with no record of hepatitis among subjects in ‘not significant stage of the disease. This may underscore the fact that opportunistic infections such as hepatitis B and C occur commonly in advanced HIV/AIDS [3, 4].

In conclusion, the prevalence of hepatitis B and C infections observed in this study is low. Consistent and deliberate preventive measures towards further reduction in prevalence rate of hepatitis B and C infections are recommended.

Conflict of Interests

The authors declare that they have no conflict of interests. All authors contributed in different aspect of the article.

References


HAEMATOLOGIC PROFILE OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED CHILDREN IN ABAKALIKI, EBONYI STATE, SOUTHEAST NIGERIA

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Abstract

Objective: Haematological abnormalities such as peripheral cytopenia in children with HIV may be manifestations of disease progression and/or side effect of drug treatment. This study was done to determine the haematological profile of HIV infected children (aged 2 to 17 years) in Abakaliki, compared to age and gender matched controls. Methods: It was a cross-sectional study that involved consecutive recruitment of 134 confirmed HIV infected children and equal number of age and gender matched HIV negative children as controls. The complete blood count (CBC) was determined using a 5-part differential automated blood analyzer. Results: HIV infected children had significantly lower mean haemoglobin (p=0.001) and total leucocyte count (p=0.011) compared to mean values of haemoglobin and total leucocyte count respectively obtained in uninfected children. Anaemia was the commonest haematological abnormality observed from the study, with 57.5% of the subjects being anaemic. A positive correlation was observed between CD4 T-cell count and anaemia (r= 0.415, p= 0.001) and between CD4 T-cell count and leucopenia (r= 0.459, p= 0.001). HIV infected children in advanced stages (3 and 4) of disease were 12 times more likely to be anaemic compared to HIV infected children in stages 1 and 2. Conclusion: In conclusion, anaemia was the commonest abnormality seen in HIV infected children and a feature of disease progression. Routine haemoglobin check is therefore recommended for all HIV infected children.

Keywords: Antiretroviral Therapy (ART), Complete Blood Count, Immunodeficiency, Haematologic Abnormality

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Introduction

Human immunodeficiency virus (HIV) infection is a major global health challenge with cases reported from virtually every country [1]. At the end of 2015, the total number of people living with HIV worldwide was about 36.7 million of this total number, about 1.8 million were children aged 0-14 years and 6.5 million of global total were in the western and central Africa [1]. Sub-Saharan Africa bore the greatest burden of the epidemic, with 68% of all people living with HIV residing in the region [2-3]. Nigeria has the largest burden of HIV in Africa, accounting for 14% of African burden. About 240,000 children are infected with HIV in Nigeria [3]. About 90% of HIV infections in children occur through vertical transmission: that is from mother to child, others are through sexual contact and exposure to blood and blood products [1-3].

HIV infection is characterized by progressive damage to the body’s immune system which results in the development of a number of opportunistic infections and other complications [2-3]. These complications may be haematologic complications such as anaemia, leucopenia, lymphopenia and thrombocytopenia [4-5]. Anaemia, leucopenia and thrombocytopenia are common haematological disorders that can occur throughout the course of HIV infection but increases with the advancement of disease [5-7]. The incidence and severity of the cytopenia generally correlates with the clinical stage of the disease, with anaemia being the most commonly encountered haematological abnormality and a significant predictor of progression to AIDS or death [6-7].

The consequences of these haematological abnormalities are twofold: firstly they can adversely alter the quality of life of the affected patients [8]. Anaemia gives rise to fatigue and dyspnea, leucopenia predisposes to infections while thrombocytopenia leads to bleeding. Secondly, these haematological abnormalities change the treatment of primary viral infection, secondary infections and neoplastic complications [8-9].

Although the incidence of haematological abnormalities can be reduced by antiretroviral therapy (ART), patients on zidovudine (ZDV) containing ART are at greater risk of developing new or worsening anaemia than non zidovudine containing therapy [10]. The presence of haematological abnormalities in HIV infected patients make treatment of such patients difficult [11]. This is not surprising as drug combination commonly used for treatment of HIV disease can also cause pancytopenia [10-11].

Adetifa et al. [12] carried out a study in Lagos, Southwest Nigeria among HIV infected children reported a high rate of anaemia (77.0%). They found a positive connection between haematologic abnormality with clinical stage of disease. There is paucity of data on haematologic profile in HIV infected children in this locality.

This study was therefore carried out to determine the haematological profile of HIV infected subjects compared with apparently normal HIV negative children. The study also sought to find out the relationship between CD4 T-cell count and haematological profile. The findings from this study may add to the increasing knowledge of this challenging disease and
may help to improve management of children with this disorder.

**Methodology**

**Study Design**

It was a cross sectional study that involved consecutive recruitment of 134 confirmed HIV infected children and equal number of age and gender matched HIV negative children as controls. The complete blood count (CBC) was determined using a 5-part differential automated hematology analyzer, (Yuesen Med, model: YSTE7501 made in China). The indices measured were haemoglobin, total leucocyte count, differential leucocyte counts and platelets. Weight and height of subjects were measured and plotted on the centre for disease control (CDC) chart. The anthropometric values of weight and height were used to classify the nutritional status of the children using the World Health Organization (WHO) weight-for-height z-score and height for age z-score (stunting). The z-scores are further classified as +1SD to > -1SD as healthy, < -1SD to > -2SD was mild malnutrition, < -2SD to > -3SD was moderate malnutrition while values < -3SD was severe malnutrition.

Stool microscopy for ova of helminth was carried out for subjects to exclude helminth infection as a cause of anaemia.

**Study area**

The study was carried out in Federal Teaching Hospital Abakaliki (FETHA). Abakaliki has a population of 141,438 [13]. FETHA operates the provider initiated HIV testing and counseling (PITC) where all children that present to the hospital are screened for HIV (using rapid tests) irrespective of their presenting complaints. Patients who tested positive are referred to the Paediatric Infectious Disease Unit.

**Study Population / Study duration**

The subjects were children aged 2 years to 17 years who have been confirmed HIV positive. Both the new enrollees (HIV infected children that were newly diagnosed) from Children Outpatient and subjects attending HIV clinics were enrolled into the study. All patients who met the inclusion criteria were enrolled between August 2015 and February 2017.

The control population (age and gender matched) was recruited consecutively from children that came for follow-up visits at children outpatient clinic of FETHA. These children would have had PITC at initial visits and were confirmed HIV negative.

**Determination of sample size**

The sample size will be determined using prevalence of anaemia (77.0%) in HIV positive children reported by Adetifa et al. [12] and prevalence rate of anaemia in controls (70.0%) by Ene-Obong and Ekweagwu [14]. A total of 134 subjects and equal number of controls were recruited during the study period. Classification of participants by their socio-economic class was determined using the classification by Oyedeji et al. [15].

Sample size determination by Charan et al. [16].
Sample Size \( (n) \) = \( \frac{r + 1}{r} \times \frac{(P^*)(1-P^*)(Z_\beta + Z_{\alpha/2})^2}{(P_1 - P_2)^2} \)

where \( r \) = ratio of control to cases, 1 for equal number of cases and control.

\( P^* \) = Proportion of cases, using prevalence rate reported by Adetifa et al.[12] of 77.0% (0.77)

\( 1-P = 1-0.77= 0.23 \)

\( Z_\beta \) = standard normal variant for level of significance as mentioned in previous section. ie standard normal variant for power of 80% which is equal to 0.84.

\( Z_{\alpha/2} \) = standard normal variant for level of significance as mentioned in previous section ie standard normal variant at confidence level of 95% = 1.96.

\( P_1 - P_2 \) = effect size or different in proportion expected based on previous studies.

\( P_1 = \) is proportion in cases (0.77)

\( P_2 = \) is proportion in control (0.70)

\( n = \) minimum sample size.

\[ n = \frac{1}{1} \times \frac{(0.77)(0.23)(0.84+1.96)^2}{(0.77-0.7)^2} \]

\[ n = 2 \times 0.1771 \times 7.84 \]

\[ = 0.0049 \]

\[ = 566 \]

However, since the sample was drawn from a finite population of HIV infected children numbering less than 10,000 a second formula correcting the sample size for a finite population was used thus:

\[ nf = \frac{N}{1 + \left( \frac{N}{n} \right)} \]

Where,

\( nf = \) Sample size for a finite population

\( N = \) desired sample size for a population more than 10,000
- The estimate of the population size which is 175 (the total number of HIV positive children with regular attendance to clinic in FETHA and Mile Four Maternity and Children Hospital Abakaliki)

Therefore, \( n_f \) the desired sample size when population is less than 10,000 will be calculated from: 
\[
\frac{n}{1 + \frac{n}{N}}
\]
where \( n = 566, N = 175 \), Thus
\[
\frac{566}{1 + \frac{566}{175}} = 134
\]

The minimum sample size was thus 134.

Minimum sample size would be 134, with equal number of age and sex matched HIV negative children for the control, bringing the total to 268.

**Inclusion criteria for subjects**

1. Children aged 2-17 years that are confirmed HIV positive
2. Selected children whose parents/guardian gave informed consent

**Inclusion criteria for controls**

1. Children aged 2-17 years who are confirmed negative for HIV
2. Selected caregivers/ guardian gave informed consent

**Exclusion criteria for subjects and controls**

1. Age less than 2 years or more than 17 years.
2. Children who have helminth infection
3. Children with symptoms and signs suggestive of pulmonary Tuberculosis
4. Children with symptoms and signs suggestive of malaria
5. Those whose parents did not give informed written consent

**Ethical considerations**

The ethical approval from the Health Research and Ethical Committee of FETHA was sought and obtained before commencement of the study. Consent was obtained from caregivers and assent from subjects that were 7 years and above.

**Data analysis**

The data obtained was entered into spreadsheet using the Microsoft excel 2007 and the analysis was done using the SPSS version 20.0. Analysis was done by the researcher and the hospital biostatistician. The significance of associations between categorical variables was tested using Chi-square and Fischer’s exact tests for comparison of proportions while Student-t test was used for comparison of means. Pearson’s correlation was also undertaken for CD4 T-cell count and haematological abnormalities. Multivariate analysis of risk factors of haematological parameters was determined using Epi Info version 7. The level of statistical significance was achieved if \( p < 0.05 \).
### Table 1(a). Age related normal haematological values (Reference ranges) [17]

<table>
<thead>
<tr>
<th>AGE</th>
<th>Haematological Parameters</th>
<th>3 months to 1 year</th>
<th>1 to &lt; 2 years</th>
<th>2 to &lt; 6 years</th>
<th>6 years to 12 years</th>
<th>&gt; 12 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>11.1 – 14.1</td>
<td>11.1 – 14.1</td>
<td>11 – 14</td>
<td>11.5 – 15.5</td>
<td>12 – 16</td>
<td></td>
</tr>
<tr>
<td>Total WBC count cells/mm³</td>
<td>6000 – 18000</td>
<td>6000 – 15000</td>
<td>5000 – 15000</td>
<td>5000 – 13000</td>
<td>4500 –</td>
<td></td>
</tr>
<tr>
<td>Absolute neutrophil count (cells/mm³)</td>
<td>1000 – 6000</td>
<td>1000 – 15000</td>
<td>1500 – 8000</td>
<td>2000 – 8000</td>
<td>2000 – 7000</td>
<td></td>
</tr>
<tr>
<td>Total lymphocyte count (cells/mm³)</td>
<td>4000 – 12000</td>
<td>3500 – 11000</td>
<td>6000 – 9000</td>
<td>1000 – 5000</td>
<td>1000 – 3000</td>
<td></td>
</tr>
<tr>
<td>Absolute Eosinophil count (cells/mm³)</td>
<td>100 – 1000</td>
<td>100 – 1000</td>
<td>100 – 1000</td>
<td>100 – 1000</td>
<td>200 – 500</td>
<td></td>
</tr>
<tr>
<td>Platelet count</td>
<td>&gt;1 month: 150,000 – 450,000 cells/mm³</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 1(b). Age - related values of certain haematological indices [18]

<table>
<thead>
<tr>
<th>Age</th>
<th>Hb/100ml</th>
<th>Haematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Birth</td>
<td>17</td>
<td>14-20</td>
</tr>
<tr>
<td>1 - 2 weeks</td>
<td>16</td>
<td>13-20</td>
</tr>
<tr>
<td>3 - 11 months</td>
<td>12</td>
<td>10-14</td>
</tr>
<tr>
<td>1 - 5 years</td>
<td>12</td>
<td>11-14</td>
</tr>
<tr>
<td>6 - 15 years</td>
<td>13</td>
<td>11-16</td>
</tr>
</tbody>
</table>
Using the two tables above, a cut-off for anaemia used in this study was haemoglobin (Hb) <11g/dl.

**Results**

Among the 134 children subjects and equal number of controls aged 2 years to 17 years who met the inclusion criteria, 56.0% were females, with female to male ratio of 1.3:1.0. Children aged 6-12 years (school age group) were the largest in number (48.5%). The median age of the study subject was 8.0 years. Majority of the subjects were from lower socio-economic class (64.9%) as shown in Table 2 below. One hundred and twenty-one (90.3%) of the 134 HIV infected subjects were on antiretroviral therapy (ART).

### Table 2. Socio-demographic variables of subjects and controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Subjects</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (n)</td>
<td>Percent (%)</td>
</tr>
<tr>
<td>Age groups (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 5</td>
<td>37</td>
<td>27.6</td>
</tr>
<tr>
<td>6-12</td>
<td>65</td>
<td>48.5</td>
</tr>
<tr>
<td>≥ 13</td>
<td>32</td>
<td>23.9</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>75</td>
<td>56.0</td>
</tr>
<tr>
<td>Males</td>
<td>59</td>
<td>44.0</td>
</tr>
<tr>
<td>Socio-economic class</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>13</td>
<td>9.7</td>
</tr>
<tr>
<td>Middle</td>
<td>34</td>
<td>25.4</td>
</tr>
<tr>
<td>Lower</td>
<td>87</td>
<td>64.9</td>
</tr>
</tbody>
</table>

The mean haemoglobin of children with HIV was low (10.2g/dl ± 3.68) compared to the mean haemoglobin of uninfected children (11.8g/dl ± 2.10) as shown in Table 3. Other haematological parameters such as total leucocyte count (TLC), platelets, absolute neutrophil count (ANC), absolute eosinophil count (AEC), absolute lymphocyte count (ALC) also had lower values when compared to uninfected children. There were significant relationships between mean haemoglobin (p=0.001) and mean TLC (p=0.011) of subjects when compared to controls as shown in Table 3 below.
Table 3. Comparison of mean values of haematological parameters of subjects with controls

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>Subjects Mean ± SD</th>
<th>Controls Mean ± SD</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>10.2 ±3.68</td>
<td>11.8 ±2.10</td>
<td>5.16</td>
<td>0.001</td>
</tr>
<tr>
<td>TLC (x 10^9 g/l)</td>
<td>6.37 ±2.88</td>
<td>7.15 ±1.57</td>
<td>3.52</td>
<td>0.011</td>
</tr>
<tr>
<td>Platelet (cells/mm³)</td>
<td>280,000 ±118,000</td>
<td>289,000 ±240,000</td>
<td>2.04</td>
<td>0.062</td>
</tr>
<tr>
<td>ANC (cells/mm³)</td>
<td>2,326 ±1,326</td>
<td>2,380 ±1,194</td>
<td>0.38</td>
<td>0.294</td>
</tr>
<tr>
<td>AEC (cells/mm³)</td>
<td>358 ±76</td>
<td>274 ±115</td>
<td>-0.79</td>
<td>0.144</td>
</tr>
<tr>
<td>ALC (cell/mm³)</td>
<td>3,183 ±1,649</td>
<td>3,412 ±1,016</td>
<td>1.87</td>
<td>0.077</td>
</tr>
</tbody>
</table>

RV= Reference value, TLC = Total leucocyte count, ANC = Absolute neutrophil count, AEC = Absolute eosinophil count, ALC = Absolute lymphocyte count

Table 4. Comparison of haematological abnormalities between HIV infected and HIV negative children

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>Reference values</th>
<th>HIV infected Below RV (%)</th>
<th>HIV negative Below RV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>11.0-16.0</td>
<td>77 (57.5)</td>
<td>18 (13.4)</td>
</tr>
<tr>
<td>TLC (10^9/L)</td>
<td>4.0-15.0</td>
<td>18 (13.4)</td>
<td>3 (2.2)</td>
</tr>
<tr>
<td>Platelets (cell/mm³)</td>
<td>150000-450000</td>
<td>16 (11.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>ANC (cells/mm³)</td>
<td>1500-8000</td>
<td>29 (21.6)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>AEC (cells/mm³)</td>
<td>100-1000</td>
<td>16 (11.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>ALC (cells/mm³)</td>
<td>1000-9000</td>
<td>9 (6.7)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>
Table 4 above shows that, 77 (57.5%) out of the 134 subjects recruited during the study period had anaemia. Of the 77 subjects that had anaemia, girls (55.8%, 43/77) had higher prevalence rate than boys (44.2%, 34/77) and children aged 6-12 years (54.5%, 42/77) were observed to be most anaemic, although no significant relationship existed between anaemia with age and gender. Eighteen subjects (13.4%) had leucopenia while twenty-nine of the subjects (21.6%) had neutropenia. Thrombocytopenia was found in 16 (11.9%) of the subjects. While normal leucocyte differential counts and platelet counts were seen in HIV negative children.

The subjects were classified into four immunologic stages (not significant, mild, advanced and severe). Majority of the subjects (89/134, 66.4%) were classified in the ‘not significant immunodeficiency stage’, 32 (23.9%) subjects were in mild immunodeficiency stage while 5.2% (7/134) were in advanced immunodeficiency stage. The subjects in stages 3 and 4 were twelve times and about thrice at risk of developing anaemia and leucopenia respectively as shown in Table 5. There were significant relationships between stages of HIV infection with anaemia (0.001) and leucopenia (0.001) as shown in Table 5.

### Table 5. Correlation between anaemia with leucopenia in HIV infected children

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Anaemia</th>
<th>Leucopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Socio-demographics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (preschool vs school age)</td>
<td>0.46 (0.05-3.96)</td>
<td>0.471</td>
</tr>
<tr>
<td>Sex (male vs female)</td>
<td>1.36 (0.34-5.44)</td>
<td>0.668</td>
</tr>
<tr>
<td>Social class (upper vs lower)</td>
<td>0.81 (0.09-7.21)</td>
<td>0.850</td>
</tr>
<tr>
<td>HIV and care</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage of HIV (stages 3,4 vs 1,2)</td>
<td>12.25 (5.67-23.08)</td>
<td>0.001</td>
</tr>
<tr>
<td>ART type (ZDV vs non-ZDV)</td>
<td>0.57 (0.11-2.95)</td>
<td>0.500</td>
</tr>
</tbody>
</table>
Discussion

Human immunodeficiency virus (HIV) through various mechanism cause peripheral cytopenia. Observation from this study showed that HIV infected children had lower mean haemoglobin, TLC and platelets count compared to uninfected children. This was similar to findings by Tagoe et al. [19] and Umar et al. [20].

HIV infection may lead to anaemia in many ways, some of which could be changes in cytokine production, decreased erythropoietin production and effects of opportunistic infections. In this study, more than half of subjects (57.5%) were found to be anaemic. This was lower when compared to the prevalence rate of 77.0% reported by Adetifa et al. [12]. This may be explained by ART use and better nutrition when compared to the study by Adetifa et al. [12]. More girls (56.0%) than boys (44.0%) were found to be anaemic and children, aged 6-12 years had the highest prevalence of anaemia (54.5%). This high prevalence of anaemia observed in these groups of subjects may be due to physiologic blood loss in girls and increased growth requirement in school age children. The HIV infected children from lower socio-economic class had a higher (58.0%) prevalence rate of anaemia compared to those from upper socio-economic class. There were no significant relationships between age, sex and socio-economic class and haematologic abnormalities among subjects in this study. This was consistent with findings by Adetifa et al. [12] who reported no relationship between haematologic abnormalities with age, sex and socio-economic class.

The mean haemoglobin level (10.2 ± 3.68 g/dl) in HIV infected children was significantly (p=0.000) lower than the mean haemoglobin level (11.8 ± 2.10g/dl) of HIV negative children. Similar to findings by Tagoe et al.[19]. The mean haemoglobin level observed in this study is consistent with that reported by Ezeonwu et al.[21] (10.4 ± 1.2g/dl) although they had a very low prevalence rate of anaemia (3.0%) due
to their low cut-off value of haemoglobin for anaemia (Hb<8g/dl). This underscores the need for an appropriate cut-off value for anaemia in children [17,18].

HIV affects directly the production of white blood cells in the bone marrow. The mean TLC of HIV infected children (6.37± 2.88 x 10⁹/l) was found to be significantly lower (p=0.011) when compared to HIV negative children (7.16± 1.57 x 10⁹/l). This is similar to findings by Tagoe et al. [19]. This study observed leucopenia in 13.4% of children infected with HIV. This is comparable to that reported by Adetifa et al. [12] (17.5%), but differed from findings by Suarez et al. [22] who noted a higher prevalence rate of leucopenia (43.0%) among subjects. Explanation for this higher prevalence rate of leucopenia reported by Suarez et al. [22] may be connected to the subjects being symptomatic and ART naïve. Coyle [23] reported neutropenia as the most common abnormality affecting the white cells of the body in HIV infected subjects. Similarly, Attili et al. [24] reported 22.7% as prevalence rate of neutropenia among subjects and was the most common white cell abnormality encountered; these were consistent with that obtained in this study where neutropenia was the most prevalent abnormality of leucocytes with a prevalence rate of 21.6%. The low prevalence rate of lymphopenia (6.7%) in index study when compared to that reported by Saurez et al.[22] and Ellaurie et al. [25] (41.0% and 43.0% respectively) may not be unconnected with the use of ART by the subjects in index study.

The mean platelet count observed in HIV infected children (281,142 ±118,000cell/mm³) was lower than that observed in HIV negative children (288,259 ±240,000cell/mm³), although not statistically significant. This was similar to the finding by Tagoe et al. [19] that reported no significant difference in prevalence rates between HIV infected subjects and controls; this was attributed to the use of ART by majority of subjects recruited. The prevalence rate of thrombocytopenia observed in this study was 11.7%, similar to that reported by Shah and Katira [26] (10.0%) while Suarez et al. [22] found a prevalence rate of thrombocytopenia to be 27.0%. Unlike the index study, Suarez et al. [22] carried out their study among symptomatic HIV patients, that may be the reason for the higher prevalence rate reported.

Epidemiological studies mainly in adult subjects, reported anaemia in subjects on ZDV containing ART combination [27,28]. Okechukwu et al. [27] reported an increased prevalence rate of anaemia with patients on ZDV combination of ART compared to non-ZDV combination. This was not corroborated in the finding of this study. Although most of the subjects on HAART were on ZDV based combination, there was no significant difference in the prevalence of anaemia between the ART types (ZDV versus non-ZDV). This was consistent with that reported by Shet et al. [29].

Anaemia is a significant predictor of HIV progression to AIDS and is independently associated with an increased risk of death in HIV infected children [30,31]. There was a significant positive correlation between CD4 T-cell count and haemoglobin level (p=0.001) in index study. This is similar to studies by Shet et al. [29], Mocroft et al. [31] and Eley et al. [32]. It was also observed in this study that subjects in advanced immunodeficiency stages (3 and 4) had 12 times higher risk of anaemia than those in stages 1 and 2. This is similar to
that observed by Kibaru et al. [33] that reported a ten-fold increase in the prevalence of anaemia in patients with advanced disease. It was therefore not surprising that all the subjects in severe immunodeficiency stage had anaemia. Also the risk of leucopenia in HIV infected children in advanced disease stages was noted to be higher than that in mild disease (stages 1 and 2), similar to that observed by Attili et al. [24].

In resource constrained countries where viral load and CD4 T-lymphocyte counts may not be done routinely or results takes a long time to obtain, studies have shown that haemoglobin level and total lymphocyte count could be used as a predictor for disease progression and a useful tool in monitoring subjects on ART [34-36].

In conclusion, this study observed that anaemia is most common haematologic abnormality and that it has a strong relationship with CD4 T- cell count. Therefore haemoglobin assay which is an affordable, accessible and available tool that can be done at bedside should be recommended for monitoring of treatment and disease progression.

Acknowledgement

I wish to acknowledge the head of Department and staff of Haematology Laboratory Unit of FETHA for their assistance in the laboratory investigations.

Limitations

All the subjects on ART were also on co-trimoxazole prophylaxis. The effect of co-trimoxazole on haematologic parameters was not determined.

Conflict of interests

The authors declare that they have no conflict of interests. All authors contributed in different aspect of the article.

References


[32]Eley BS, Sive AA, Shuttleworth M, Hussey GD. A prospective, cross sectional study of anaemia and peripheral iron status in


INTRODUCTION AND OBJECTIVE: Pneumococcal disease is a leading cause of morbidity and mortality worldwide. There were limited publications on invasive pneumococcal infection (IPD) in Malaysia. The aim of this study is to describe retrospectively cases of IPD in hospitalised children of less than 12 years old and highlighting the unusual cases. Methodology: A retrospective review of children with IPD from March 2002 to November 2005 at a tertiary paediatric hospital. IPD cases were defined as isolates of *Streptococcus pneumoniae* from a normally sterile body fluid site. Results: Twenty-four patients were identified with a male preponderance. Two-thirds of patients were below 1-year-old; with three cases presenting in the premature newborn. Thirty-seven percent of cases had underlying conditions. Sepsis and pneumonia were the commonest manifestation, followed by meningitis. The unusual manifestations were in a form of post-infectious glomerulonephritis and overwhelming purpura fulminans. There were two mortalities; both infants had meningitis. Antibiotic susceptibility pattern showed that more than half of the isolates were sensitive towards penicillin and erythromycin. Penicillin resistance was found in 6 (25%) isolates. Conclusion: IPD results in significant morbidity and mortality, especially in young children below 2 years of age and justifies further evaluation of preventive strategies including the implementation of pneumococcal vaccine in the national immunisation programme.

Keywords: Invasive Pneumococcal Disease (IPD), *Streptococcus Pneumoniae*, Children, Unusual

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Fax: +603-89489369
Email: ijah@upm.edu.my
Introduction

*Streptococcus pneumoniae* remains an important bacterial cause of invasive infection in children worldwide. The World Health Organization (WHO) estimates that 1.6 million die every year from the disease, 0.7-1.0 million of which are children aged <5 [1]. The morbidity and mortality associated with this disease remain high despite monumental advances in medical technology. In Malaysia, several reports have shown an increase in antibiotic resistance over the past few decades. The rate of non-susceptible penicillin was noted from 2% in 1988 to 50% in 2011. Moreover, since the introduction of *Haemophilus influenzae* type b in 2002 in Malaysia, *S. pneumoniae* has become a predominant pathogen in bacterial infections, especially meningitis, in children [2, 3]. As drug-resistant organisms continue to emerge worldwide, more unusual pneumococcal infections are seen. There were limited publications on invasive pneumococcal infection (IPD) in Malaysia, as previous publications were confined on antibiotic susceptibility and serotyping of the disease. The purpose of conducting this study was to describe the clinical manifestations and outcome of children with IPD admitted to a tertiary hospital and highlighting the unusual cases seen in the cohort of patients.

Materials and Methods

Subjects

This was a retrospective study conducted in Institute of Paediatrics, Hospital Kuala Lumpur (IPHKL), a tertiary paediatric hospital with 300 beds, which provided both primary and tertiary health care for children. The study period was from March 2002 to November 2005. The medical records were reviewed to obtain demographic data and clinical information. A proforma sheet was used to record baseline information, including socio-demographic characteristics of family, breastfeeding, co-morbidity conditions and antibiotic administration within 30 days before admission. Inclusion criteria were as follows: age at recruitment less than 12 years and children with positive culture for *S. pneumoniae* from normally sterile body fluids. Patients with coinfections were excluded, however, a newborn with congenital syphilis was included in this study for the purpose of illustrating an unusual presentation of IPD in this age group.

Sample Size Calculation

The calculation for the study sample size was based from the largest population in Gambia who were severely affected by the disease [4] with a prevalence of 250/1000000 children. Taking into account of the true population ratio, a confidence interval of 95% and an assumption rate of 2.5% of the estimated affected Malaysian paediatric population, a precision single proportion sample size was calculated. The calculated sample size of 37 children was sufficient to meet the study objective.

Case definition

A case of confirmed IPD was defined in accordance with the Centers for Diseases Control and Prevention (Atlanta, GA, USA) as isolation of *S. pneumoniae* from a normally sterile body fluid site [5]. Pneumococcal septicaemia was defined as presence of the organism in the blood with clinical features of sepsis that fulfilled the international paediatric sepsis consensus as outlined by Goldstein [6]. The diagnosis of pneumococcal pneumonia was made with presence of positive culture of the organism in blood or pleural fluid compatible with a
clinical manifestation of pneumonia (fever with tachypnea or chest retraction or signs of lower respiratory tract infection e.g. crepitation or reduced breath sounds) supported by a radiographic evidence if available. For meningitis, according to the CDC guidelines [7], the diagnosis of a definite case required the isolation of \textit{S. pneumoniae} from the cerebrospinal fluid in a patient with clinical syndrome consistent with the diagnosis of bacterial meningitis (e.g. fever >38.5°C, headache, neck stiffness, altered consciousness or other meningeal signs). A probable case was defined as the association of a compatible clinical syndrome with an abnormal cerebrospinal fluid examination (leucocytosis, elevated protein or low glucose) in the presence of a positive blood culture for the organism. On the other hand, finding of the isolate in the blood culture alone in such a clinical scenario was considered as presumed meningitis. The diagnosis of septic arthritis was confirmed with presence of \textit{S. pneumoniae} in blood or joint aspiration. Pneumococcal peritonitis was diagnosed when there was evidence of peritonitis with the isolation of \textit{S. pneumoniae} from blood or peritoneal fluid.

\textbf{Identification of isolates and antimicrobial susceptibility testing}

The presence of \textit{S. pneumoniae} isolates were identified by colony morphology, Gram-staining and catalase reaction. It was further confirmed by optochin sensitivity (> 5 mm inhibition) and bile solubility by using standard microbiologic methods according to guidelines from the Clinical and Laboratory Standards Institute (CLSI) recommendations [8,9]. The antibiotic susceptibility pattern of the isolates was determined by the standard disc diffusion method.

\textbf{Statistical analysis}

Data were keyed in and analysed using Statistical Package for Social Sciences Version 19.0 (SPSS Inc., Chicago, IL, USA). Descriptive variables were calculated as percentage and mean.

\textbf{Results}

\textbf{Demography and clinical characteristics of patients}

During the study period from March 2002 to November 2005, data of 24 patients who had satisfied the inclusion criteria was included. The results showed a male preponderance, with a 2:1 distribution (sixteen male vs eight female). The mean age of presentation was 17.9 months (ranging from newborn to 77 months). More than two-thirds of the patients (1/24, 70.8%), were below 2-year-old. Three patients were newborn and presented as early as within the first 24 hours of life. All the infected newborn had positive yield of the organism from blood, were also ex-premature babies. One had a vertical transmission as confirmed by positive maternal high vaginal swab and another had a superimposed congenital syphilis. Slightly more than one third of patients (9, 37.5%) had co-morbid risk factors identified. Four patients had haematological conditions (one acute lymphoblastic leukemia and three thalassaemia), three prematurity and the remaining two were associated with nephrotic syndrome. Only three patients received antibiotics (12.5%) within 30 days of admission. Mean duration of illness before admission was 4.9 ± 3.9 days. However, the information was not available for five cases.
Clinical manifestations on presentation

Table 1 shows the clinical manifestations of IPD in the 24 patients. Sepsis and pneumonia were the commonest manifestation with equal numbers of patients diagnosed in each group of clinical syndrome. This was followed by 10 (41.7%) patients with meningitis.

Table 1. Clinical Manifestation of Invasive Pneumococcal Disease

<table>
<thead>
<tr>
<th>Clinical manifestations</th>
<th>Number of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepsis</td>
<td>14</td>
<td>50.0</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>14</td>
<td>50.0</td>
</tr>
<tr>
<td>Meningitis (total)</td>
<td>10</td>
<td>41.7</td>
</tr>
<tr>
<td>Definite</td>
<td>4</td>
<td>16.6</td>
</tr>
<tr>
<td>Probable</td>
<td>1</td>
<td>4.3</td>
</tr>
<tr>
<td>Presumed</td>
<td>5</td>
<td>20.8</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>3</td>
<td>12.5</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>2</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Unusual manifestation

Acute glomerulonephritis was one of the unusual presentations in our study. The patient developed features of acute glomerulonephritis following pneumococcal sepsis and cervical lymphadenitis. The laboratory studies were in keeping with glomerulonepritic picture; and blood-culture yielded *S. pneumoniae* The other was a case of purpura fulminans (PF), presenting in a previously healthy infant. He presented with meningococcal-like manifestation in septic shock. He was discharged well without any gangrenous sequelae of the affected extremities.

Outcome

The majority of patients required prolonged hospitalisation. Only ten (41.7%) were discharged within one week of hospitalisation. Seven patients required admission for more than four weeks. Thirteen patients (54.2%) required ventilation, ranging from 1-24 days duration.

Three patients developed pleural effusion or empyema, with one patient requiring chest tube drainage. Other complications following meningitis were subdural effusion (3 patients) and hydrocephalus (2 patients). There were two mortalities; both infants

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(aged four and seven months) had meningitis.

**Antibiotic susceptibility and resistance pattern**

Figure 1 illustrates the susceptibility to antibiotic and culture sensitivity. However there were inconsistencies on the type of antibiotics tested as shown in the figure. A total of 11 antibiotics were tested; however only penicillin, erythromycin and ceftriaxone were consistently being tested. By using oxacillin disc diffusion method, it was shown that more than half of the patients (54.2%) were still susceptible to penicillin. Penicillin resistance was found in 6 (25%) isolates. Erythromycin susceptibility was found in 15 patients (62.5%) while five patients (20.8%) had resistance. Two isolates (8.3%) were—non-susceptible to ceftriaxone.

![Figure 1. Antimicrobial susceptibility of S. pneumoniae isolates](image)

**Discussion**

There were limited clinical studies on childhood IPD in Malaysia; most of the previous studies focused on the microbiological aspect of the disease. The introduction of pneumococcal conjugate vaccines (PCV) has successfully reduced the incidence of vaccine-type IPD. Since March 2006, the vaccine has been licensed in Malaysia. However, it has not been included as part of the routine vaccination program in Malaysia despite it being projected to prevent around 260,000 deaths annually as well as having the potential to mitigate the widespread antibiotic resistance [10, 11]. This data is important as it highlights the severity of the disease in the pre-vaccination era in Malaysia.

More than two-thirds of our study population were aged < 2 years. Most of the patients were infants. Susceptibility to IPD in association with extreme age in children is also reported in other studies worldwide [12,13]. Children aged < 2 years are 5 to 10 times more likely to contract an IPD than the rest of the population. This may be explained by the various risk factors such as the relatively high frequency of
nasopharyngeal infection and colonisation, the immature and developing immune system in this susceptible aged group, the likelihood of cross-infection in a child care centre and low-socioeconomic status [14]. Boys seemed to be more likely to be infected than girls; this higher prevalence in males was also reported by other authors [15,16].

Published studies on pneumococcal infection in Malaysia have shown pneumonia to be its most common clinical presentation [10,15]. However, in this study the more common manifestation of IPD were sepsis and pneumonia. The explanation for the overlap probably could be due to the non-specific presentation manifested in both the clinical syndromes and the diagnostic criterion of a positive culture for both the clinical syndromes especially in early childhood. On the other hand, meningitis, the most serious manifestation of IPD, was the cause of the two fatalities in this study, giving rise to a fatality rate of 8%. The global pneumococcal meningitis case-fatality rate and morbidity are known to be high. The case fatality rate is estimated to be 59%, ranging from 29% in western Pacific to 73% in Africa [12].

Pneumococcal sepsis is rare in newborns. We reported three cases of newborns with pneumococcal sepsis and all of them shared the same risk factor, which was premature birth. The reported incidence of this unusual cause of sepsis in newborn ranged between 1-11% in contrast with 0.18%- 0.75% in vaginal cultures, implicating a high invasion rate of the organism in newborn [17,18]. The clinical course strongly resembles early onset group B streptococcal disease with higher mortality and morbidity rate. The reported mortality rate was as high as 20-60% [19,20], especially in patients presented within 48 hours of life (early onset sepsis). In view of the high invasion rate and potential disastrous outcome, treatment of the asymptomatic newborn colonised by S. pneumoniae has been recommended [19]. Since the introduction of PCV, the rate of IPV in neonates caused by vaccine serotypes has decreased significantly, although the recommendation for the vaccine is not until the infants reach 2 months of age [20].

Unusual manifestations of pneumococcal infection incidence has escalated in the era of increasing resistance of S. pneumoniae worldwide. In our case series, we highlighted the rare manifestations in the form of post-infectious glomerulonephritis and an overwhelming PF. Post-infectious glomerulonephritis is commonly seen as a complication of infection with nephritogenic strains of group A β-haemolytic streptococcus, mostly with Streptococcus pyogenes. On the other hand, S. pneumoniae is an unusual organism leading to glomerulonephritis. Pneumococcal serotypes described as nephritogenic are 5, 7, 9, 14, and 15 [21]. The occurrence of nephritis is about 3 – 10 days after the primary infection which is often pneumonia. It is postulated that deposition of immune complex is associated with glomerulonephritis and pulmonary alveolitis [22]. As in other children who had acquired pneumococcal glomerulonephritis, the resolution of nephritis in our patient was after 4 weeks.

The other rare presentation reported was in the form of an overwhelming pneumococcal sepsis with PF [23]. Most cases of PF caused by S. pneumoniae infections occur in adult population and in asplenic or immunocompromised patients. Pneumococcal-associated PF in immunocompetent children is extremely rare, as in our patient. Even though pneumococcal-associated PF in immunocompetent children is rare, it should
be considered especially in children without history of pneumococcal vaccination.

Slightly more than one third of our patients had co-morbid risk factors identified. A study on co-morbid conditions showed that children with IPD had increased rate of hospitalisation, morbidity and mortality compared with the general paediatric population [22, 23]. The highest risk was observed in children with primary immunodeficiency and haematological cancer. Unfortunately, missed opportunities for pneumococcal vaccination coverage were found rampant among high-risk children as illustrated in an Italian study where the uptake of the vaccine in these children with HIV infection, cystic fibrosis, liver transplantation or diabetes mellitus was low [26]. Thus, there is a need for education of healthcare professionals, patients and families regarding the importance of vaccination in at-risk children.

The results of this study were consistent with other pre-antecedent studies. However, as this was a hospital-based study with small sample size, the severity and manifestations of the disease were not representative of the true incidence in Malaysia. To estimate IPD burden among children in Malaysia, a prospective study involving the collaboration of multiple centers with routine performance of blood culture in all febrile children in both in-patient and out-patient setting is required.

Limitation: As this was a single centre study within a short period, there was difficulty in obtaining adequate number of subjects. The retrospective nature of the study could also contribute to an inadequate sample.

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References


CASE REPORT

MUSIC THERAPY AND HOSPITALIZED CHILDREN: THREE CASE STUDIES

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Abstract

Music therapy is rarely available within the medical services in Malaysia. Hospitalized children experience a great deal of stress, anxiety, and pain due to unfamiliar environment, separation from the family, a variety of medical procedures and treatments, and the uncertainties of the illnesses. Music is often used to cope with both physical and psychological distress clinically because of its pervasiveness and familiarity. The purpose of this article is to elaborate the effectiveness of music therapy in addressing both physical and psychosocial needs of hospitalized children. The three case studies demonstrated how a variety of music therapy interventions are used to facilitate individualized goals and to improve the quality of life.

Keywords: Music Therapy, Music and Pain, Hospitalized Children, Psychological Distress

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Introduction

Children face various types and levels of challenges when undergoing treatments and procedures in medical settings that can cause them physically and mentally distressed. They experience stress, pain, and trauma, as a result of, but not limited to, hospitalization, surgery, separation from their family and home, or even loss of limbs. In some Western countries, music therapy is widely used in medical settings, especially within pediatric units. Research has shown the significance and effectiveness of music therapy used within medical settings [1-5]. Among hospitalized individuals, one of the main tenets experienced is pain. Gutgsell et al. examined 200 palliative care adult inpatients who did not differ according to gender, mean age, ethnicity, and baseline pain severity. The music therapy group was given a single music therapy intervention that incorporated both guided-autogenic relaxation and live music. Although both the music therapy and the control groups showed significant decrease, the music therapy group displayed a significantly greater change on the pain numeric rating scale. The music therapy group also showed
a significant decline in the functional pain score [3].

Magill reviewed the potential benefits of music therapy based on the pre- and post-treatment scores of 90 patients at a cancer centre. The data indicated improved scores in different aspects such as pain, fatigue, anxiety, depression, and nausea. In addition to the benefit for the patients, the investigator also found the effectiveness of music therapy in enhancing the well-being of family members as well as of the staff at the cancer centre. The effects may be immediate or long lasting, from addressing the physical discomfort to the mental distress, and eventually leading to the ultimate goal – improving the quality of life [4].

With a lack of or insufficiency in verbal skills, children may experience difficulty in communicating pain. When words become a barrier, children may display their discomfort through emotions and behaviors. From simply frowning, wincing, sobbing, whining, crying, to yelling, screaming, kicking, punching, hitting and other behaviors exhibited by children; healthcare workers and caregivers may experience different levels and challenges of these exhibited behaviors before, during, or after treatment.

Barrera, Rykov & Doyle investigated sixty-five hospitalized children age ranged from six months to seventeen years old at a child oncology unit. The study revealed that children showed significant improvement in feelings of comfort after music therapy intervention. The parents reported that while music therapy was helpful in providing comfort to their children, they experienced less anxiety [5]. Longhi & Pickett examined the physiological responses of a total of twenty-one long-term hospitalized children aged from three months to fourteen years old when exposed to live music. Although the heart rate measured did not change significantly, the participants’ oxygen level in the blood increased significantly. The researchers suggested that music may potentially improve the children’s physiological and psychological well-being [6].

According to the World Federation of Music Therapy, music therapy is an allied health profession in which music and its elements as an intervention in medical, educational, and everyday environments with individuals, groups, families, or communities who seek to optimize their quality of life and improve their physical, social, communicative, emotional, intellectual, and spiritual health and wellbeing [7]. Music as a non-threatening, pervasive, and flexible medium, can be structured accordingly to provide a strong and safe fundamental basis for individuals to experience and explore.

Music therapy is a relatively new profession in Malaysia, and is currently a service mainly in the area of children with special needs. In year 2012, music therapy was first introduced to a local medical setting in the Klang Valley. A music therapist was hired three hours per week to work at a pediatric ward. Due to a lack of awareness, the music therapist needed to approach the patients individually most of the time and occasionally, patients were referred by the physicians; and rarely were patients self-referred. Though music is universal, the selection of music is crucial in a therapy process. The rationales contributing to the selection of music used in therapy is age, cultural difference, personal experience, religion, musical background, and music preference. In the multicultural context of Malaysian society, the diversity of music is
both an asset and a challenge to the music therapy community. With the latest technology, music is easy to access. Patients have a variety of musical genres to select their favorites. In addition to the musical genres, patients listen to songs in other languages, albeit they may not understand the language. Despite the importance of patients’ music preferences in a therapy process, music therapists must consider other imperative justifications when selecting music. These justifications are inclusive of, but not limited to cultural background, religion, ethnicity, and education background. Thus, a music therapy assessment is essential before the therapy starts. Besides assessing a patient’s functioning levels in several domains namely physical, cognitive, communication, behavioral, emotional, social, and psychological, it is important for a music therapist to assess the patient’s musical responses and take note of his or her music preference and musical background. Regardless of the musical background, a patient’s interest in music may signify a greater benefit he or she may gain from music therapy. This article consists of three case studies that demonstrate how different music therapy interventions have helped each individual to cope with both their physical and psychological distress during hospitalization. The age range of the individuals in these case studies is between one and seventeen years old.

Case 1: Baj

Suffering from Cystic Fibrosis, Baj, a 17-year-old boy, was referred for music therapy by a physician to enhance his verbal expression. According to the physician, Baj rarely communicated his needs, and had difficulty in making decisions. Another main reason of the referral was because the physician saw him playing the guitar when he was hospitalized. This showed that Baj had interest in music and music therapy might be beneficial for him in addressing his needs.

During the assessment, the therapist observed many indifferent responses from Baj. He was afraid to make a choice, from simply choosing a musical instrument to deciding what to eat for lunch. Even if a choice was made, Baj tended to retract his words. Baj’s verbal responses to any conversation attempts by the therapist were general and brief, thus reflecting his referral for music therapy. He did not identify any personal feelings and only commented “nice” and “good” when asked. In the first two music therapy sessions, Baj sang the songs the therapist brought to him and for the songs he requested, he would play the guitar and sing along. Baj displayed more participation in music than in conversation with the therapist. When discussing any feelings brought up by the songs, Baj avoided exploring further, quickly moving on to the next topic.

In the third session, Baj was reluctant to sing or play any instruments due to diarrhea. However, he wanted the therapist to stay and talk to him. Baj displayed curiosity towards the glockenspiel, an instrument he had never seen. The therapist proposed for Baj to play his name on the glockenspiel. Displaying enthusiasm to play the glockenspiel, Baj sat up from a lying position and after some exploration, expressed his wish to compose a “nice and personal” song. He shared with the therapist about his daily interactive experiences between himself and his mother. He joked that “N” and “O” were the two most frequent letters in his life as “No” was the most common response from the mother. Besides “No,” Baj also mentioned that “no need” was his favorite phrase in responding to his parents. As he said, “they always said...
no, and the next thing they do is to give me an alternative plan; so I will tell them ‘no need’.” To Baj, those alternative plans acted like a consolation to compensate what he wanted to do, which he felt disgusted.

The therapist gathered some keywords such as “no,” “no need,” “cannot,” for Baj to begin writing the lyrics. He wanted to stress the significance of the letters N and O. He did show some frustration by sighing and claiming that it was hard in the beginning stage of composing but his determination of composing the song was strong. For the melody, Baj chose four notes for the melody, as a metaphor to reflect his life – “dull, limited, and boring.”

Verse 1:

Genting theme park my dream place
But but but you always say N O no
N O no to congested place
N O no to cable car
N O no to roller coaster
N O no to playing with water
N O no to staying overnight
N O no to smoky places
And yes to staying at home
And I must wear a mask
And I must wear a mask

Verse 2:

These are my favorite food
But but but you always say N O no
N O no to oily food, goreng pisang
N O no to cold drinks, vitagen
N O no to curry food, laksa
N O no to spicy food, pepper
N O no to sweet food, ice-cream
N O no to gas drinks, Sprite
And yes to plain water
And I must take the medicine
And I must take the medicine

Chorus:
N O no means cannot
CANNOT cannot means no need
CANNOT cannot means no need

Figure 1. Baj’s Sad Song
Upon completion, Baj initiated to sing and play the song. He expressed his wish to have the song recorded and played during his funeral. Baj discontinued music therapy sessions immediately after the third session, citing personal reasons. The songwriting intervention fostered a variety of therapeutic aims for Baj: reflection of his past, present, and future; self-expression; and support for coping and adaptation. The lyrics were what he experienced in the past and present while at the same time, Baj was hoping that no alternatives would be offered in future. Even though the lyrics depicted the forbidden food and actions that implied a negative circumstance, Baj used major key and mostly major chords indicating a brighter and positive sound in his composition. As he aptly stated, “I have a sad life but my song does not need to be sad.” Out of the many unpleasant experiences, Baj found a way to support with his sadness through music therapy. Despite the limited sessions, music provided a safe fundamental for Baj to express his life and his perspectives about his life. His verbal expression and sharing increased during the songwriting process.

Case 2: Amy

A shy and quiet adolescent girl, 14-year-old Amy was soft spoken and passive when the therapist met her. Suffering from leukemia and undergoing bone marrow transplant, Amy was in the isolation unit alone with her elder sister as a caregiver. She looked bored and passive as the therapist approached her in the isolation room. During the assessment, Amy claimed that she enjoyed listening to music, both Malay and English pop songs. However, she was shy to sing as she felt that she did not have a good voice. The therapist observed that Amy was uneasy when the sister was not around. She avoided eye contact, nodded or shook her head to respond, and sometimes answered very briefly to the questions asked. The therapist aimed to engage Amy in music therapy, hoping to help her in expressing herself and enlighten her life in the isolation room.

In the first session, the therapist brought a songbook to Amy and she browsed through with excitement when she saw the songs she knew as evidenced by whispering to her sister, raising eyebrows, and her smiles. Amy picked several songs but asked the sister to sing. With much encouragement, she finally agreed to sing with the sister while the therapist accompanied them on guitar. Yet, her voice was barely heard. After a week, Amy was delighted and excited to see the therapist again. The sister claimed that Amy was so contented after the previous session and kept looking forward to the second session. In the following two sessions, Amy became more comfortable with the therapist and they sang songs for most of the time. Her verbal expression increased and she sustained longer eye contact.

In the fourth session, Amy requested a song Takut (Fear) by Amalia Alias.

Mungkin tak bisa menyatakan
Apa yang terlintas di benakku
Bukan kerana ku tak mencinta
Cuma kerana ku tak mampu menatap matamu
Tapi bukankah semua rasaku

(Maybe I am unable to express)
(What is in my mind)
(Not because I do not love)
(Just because I cannot look into your eyes)
(But it’s not all my feelings)
Amy requested to sing this song repetitively. The therapist asked what stood out in this song and Amy pinpointed the simplicity. Without any instrumental accompaniment, Takut (Fear) is a monophonic song that made Amy feels closer to God. She described the feeling as whispering to God in complete silence. Amy acknowledged that the song expressed how she felt - “I want God to help me. I don’t want to be alone here. I’m scared to lose myself.”

Amy’s expression of the word “scared” was a breakthrough. The acknowledgment of the scared feeling was important for Amy to connect to her inner self and realize how the scary feeling haunted her constantly. While sobbing, Amy asserted that she was constantly feeling scared, from the moment after being diagnosed to the time when the treatment started. “I don’t know what the doctor or the nurse is going to do next because they only told my parents. When I am allowed to go home, I am scared that this might be the last time being at home. It’s like every action I take, I feel scared, I don’t know what it may cause… and now, I don’t know what is next. Sometimes I think I am even scared of the time. I don’t know what is going to happen in the next minute, next second,” Amy disclosed.

With the fear of uncertainty due to a loss of sense of control, Amy felt insecure. The song, Takut, portrayed the fear and insecurity, served as a bridge for Amy to connect to her inner self, and a means of emotional expression. She verbalized the connection after repeatedly singing the song. The use of songs in the isolation room has potential to re-establish human contact and enhance communication [8]. With no referral made but observing her needs, the therapist’s goal for Amy to express herself was attained and her involvement in music therapy increased as evidenced by more participation, sustained eye contact, and more comfortable in sharing her thoughts verbally.

Aldwin cited seven major adaptive tasks originally proposed by Moos and Schaefer - dealing with psychological consequences of the illness, dealing with the treatment and hospital environment, and developing and maintaining good relations with health care workers, are classified as “illness-related tasks” while the other four – maintaining emotional equilibrium, a sense of self, good
relations with family and friends, and preparing for future exigencies, are described as “general tasks” [9]. In Amy’s case, her fear was at first caused by the diagnosis, followed by the treatment, and none of the fears was addressed until it swelled and triggered her coping mechanism to suppress, later to hide, and finally refused to feel. Her “illness-related” issues affected her emotional being, causing her to be deficient in self-competence and social interaction. Both “illness-related tasks” and “general tasks” intertwined.

In Amy’s case, music was used and intended to lessen the feelings of threat in an unfamiliar environment. In her article about the use of songs in treatment of oncology patients, Dileo stated that lyrics allow one to project his or her feelings in a secured context. Songs carry important messages and meanings, and stimulate communication by bringing insight into one’s feelings and thoughts [10]. According to Kallay, music seems to play an important role in an adolescent’s life as it comprises socialization, coping strategies, emotional expression, and individualism [11]. With the song *Takut*, Amy found a means to revisit her fear, re-experience it, and subsequently, externalize it through verbal expression. The song discussion process enabled Amy to get connected with her inner self within a non-threatening context and therefore, she was able to risk the experience of feeling the fear. Her acknowledgement of the fear allowed her to consequently learn to cope and adapt.

**Case 3: Kay**

At the age of one, Kay was referred to the therapist due to the issue of food intake. She suffered from Hirschsprung’s Disease and because of many complications, doctors seemed unable to define the main cause of her refusal of food intake and suspected that the contributing factor was mainly psychological barrier. The referral came from Kay’s mother as she was unable to get any other professional help from the facility.

Kay had been staying in the hospital from birth, lies on the bed for most of the time, and received minimal stimulation. Therefore, her overall development was slow – light weight, clumsy movements, unable to suck, brief eye contact, and no vocalizations. Sometimes, Kay cried and threw tantrums without any apparent reasons. Long duration of hospitalization may lead to an interruption of a child’s development due to the unstimulating environment [12]. As Kay was unable to suck, the mother usually fed her milk by syringes. When Kay saw the mother was preparing milk, Kay started to frown, used her hands to cover her face, and sometimes even buried her head under the pillow. The mother claimed that the feeding time was the most stressful moment for both of them. She described it as a tug of war in which Kay struggled to the maximum to avoid the syringe and as a mother, she used up all her strength to catch and feed her. At times, after feeding, Kay induced vomiting herself and the mother needed to feed again. The mother also admitted that sometimes she would lose her temper and break down.

After the assessment, the therapist paired feeding and music to promote a pleasant experience as well as using music as a reinforcer. To engage children in musical interaction, it is essential to ensure that the familiarity of the repertoire [13]. Before the feeding, the therapist began to sing a couple of Kay’s familiar children songs and play instruments with her, intending to distract her from seeing the mother preparing for milk. When the milk was ready, the therapist would start singing an adapted Chinese children song. The lyrics were changed to adapt to the feeding time with the
instructions of opening the mouth, chewing, and swallowing, and reinforcements such as “well done” and “good job” included in the song. Kay seemed to respond to the adapted song very well by following the instructions while making good eye contact with the therapist. The therapist also structured a reward of instruments’ playing time for Kay after eating. This accelerated the pace of feeding from 30 minutes to 15 minutes as Kay looked forward to playing with the instruments. She did not display any self-induced vomiting after feeding as she focused on playing the instruments. Music served as an effective distraction in this scenario.

In addition to pairing music with feeding, music is used to support Kay’s overall development, inclusive of but not limited to communication, cognitive, and emotional wellbeing through normalizing the environment. On the eighth session, Kay began to show interest in vocalizing. She initiated to vocalize “gee-ta” when she saw the therapist coming in with a guitar. Two months later, Kay was able to verbalize “don’t want” and shook her head at the same time to express her dislike when the therapist introduced a harmonica. However, after several sessions, Kay was captivated by the therapist blowing the harmonica and eventually, Kay learned how to blow and suck using the harmonica. In addition, her imitation skills improved tremendously as she always imitated the therapist in playing a variety of instruments. For instance, she played a glissando on the glockenspiel after the therapist played once; and she used her fingers to play the djembe after observing the therapist. These music experiences, according to Loewy, which involve esteem building and motivation, may aid in the appetite recovery and make the intake of food as a pleasant and positive experience [14].

During Kay’s hospitalization, she suffered from diarrhea that caused some infection around her anus area. This was extremely painful whenever the mother changed her diapers. She screamed, cried and refused to change. The therapist used her favorite song – The Wheels on the Bus - to help her in dealing pain. To entrain her screaming and crying, the therapist incorporated her breathing and crying pace with her tone of crying in the song. Besides her high pitch screaming, the therapist also incorporated her behaviors in the song. The song served as a situation song, which meant to comfort, reassure, and show acceptance; and eventually to regulate Kay’s emotional state. The focus of a situation song lies on the here-and-now and the therapeutic relationship [15].

(Kay) in the room goes pain pain ah
Pain pain ah x2
(Kay) in the room goes pain pain ah
All day long
(Kay’s legs) on the bed go kick kick kick
Kick kick kick x2
(Kay’s legs) on the bed go kick kick kick
All day long

Figure 3. Adapted “The Wheels on the Bus”
When Kay first heard of the adapted song, she stopped her crying and immediately turned her head towards the therapist. She paid full attention to the therapist’s singing and guitar accompaniment and even smiled. Kay’s pain was distracted. This can be explicated by the figure-ground relationship under Gestalt theory. Pain was the figure and yet when music emerges, pain recedes to the background while music becomes the figure. By utilizing the song to assist Kay in dealing with pain, the mother claimed that the changing time became easier and less stressful.

Music therapy supported Kay for nearly two years, from the first encounter of food refusal, to the later stage of pain management, and throughout her overall development. Kay benefited remarkably from music therapy; as her physical and developmental needs were addressed and as a result, her mother’s anxiety and fatigue levels also decreased.

Conclusion

The three individuals in the case studies comprise the three main ethnicities in Malaysia – Malay, Chinese, and Indian. The songs used in the case studies encompassed all three main languages – Malay, Chinese, and English - used in Malaysian society. As for their musical background, only Baj displayed adequate guitar skills. Both Baj and Amy showed interest in music during the assessments. For Kay, she was not exposed much to music before the therapy began. However, their experiences in music therapy demonstrated that the significance of music therapy regardless of their ethnicity, religion, musical skills or their exposure to music.

The three case studies provided a clear picture of how the maximized use of music in medical settings, particularly with hospitalized children, is beneficial. Though unable to continue the therapy process, Baj had a session which allowed him to reflect his past experiences, and express his helplessness and disappointment. For Amy, a simple song enabled her to acknowledge a genuine feeling she had long suppressed. Her attention to the feeling of fear was significant as emotional expression is taken as a crucial element in the music therapy process as the suppression of expression is associated with the susceptibility to and survival from cancer [11]. From refusing to take in any food to the pain caused by the infection, the flexibility of music was seen greatly in Kay’s case. Music was used as a reinforcer to reward her food intake, a sensory stimulation for her speech, a tool for her overall development, as well as a distraction and integration in helping her to cope with pain. All three case studies demonstrated how music therapy addressed different areas of needs, from physical goal as in pain management, developmental goal inclusive of developing expressive language and cognitive functioning, to psychosocial needs that focus on self-expression and emotional support.

These case studies demonstrated the effectiveness and advantages of using music in medical settings. As a non-threatening and pervasive medium, music provides a safe context and simply connects with patients. Under allied health, music therapy is equally important as other professions such as physical therapy, occupational therapy, speech and language therapy, and recreational therapy, in delivering direct and professional patient care. In Malaysia, music therapy has not been widely used in patient care in medical settings. With its effectiveness, music therapy is undoubtedly a valuable and beneficial alternative therapy for patients who undergo different levels of
physical and emotional distress. With the overarching emphasis on holistic treatments globally, the collaboration of mainstream and conventional treatment with alternative therapies is gaining more attention and has received its recognition [16]. Hence, music therapy is strongly recommended in local medical settings to support patients both physically and mentally.

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